出版者
農業部農業試驗所

Published by
Taiwan Agricultural Research Institute,
Ministry of Agriculture

ISSN 2790-086X
9 772790 086007

台灣農業研究

Journal of Taiwan Agricultural Research

Vol.75 No.2 2026

ISSN 2790-086X
DOI:10.6156/JTAR

Vol.75

台灣農業研究

Journal of Taiwan Agricultural Research • No.2 2026

台 灣 農 業 研 究

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):171–184 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0001

Feature Article

Current Status of Edible Insect Industry in Korea: Contemporary Issues, Challenges, and Path Ahead

Sampat Ghosh[1,2], Sukjun Sun[3], and Chuleui Jung[4,5,*]

Abstract

Ghosh, S., S. Sun, and C. Jung. 2026. Current status of edible insect industry in Korea: Contemporary issues, challenges, and path ahead. J. Taiwan Agric. Res. 75(2):171–184.

The edible insect industry in South Korea has evolved from its deep-rooted tradition of using insects such as silkworm pupae for food and medicine to a modern sector integrating advanced technologies and sustainability. Recent years have seen significant expansion, with approximately 245 operational insect farms producing species such as mealworms, crickets, silkworm pupae, black soldier fly larvae, and honeybee drones. Supported by regulatory frameworks like the Insect Industry Promotion Act (2011) and Hazard Analysis and Critical Control Point (HACCP) certification, the industry aims to address food security, rural development, and environmental concerns. Large-scale production facilities and AI-driven automation have increased efficiency, while global collaborations and export strategies have positioned South Korea as a leader in the global edible insect market. Nevertheless, scaling up production raises sustainability challenges, especially regarding energy use and carbon footprints. To maintain environmental benefits, strategies including renewable energy adoption, circular economy practices, and innovative processing are essential. Consumer acceptance is fostered by integrating insects into familiar food products and animal feed. Overall, South Korea's edible insect sector represents a fusion of cultural heritage and cutting-edge technology, contributing to food security, sustainability, and health. The industry's continued growth will depend on balancing large-scale production with environmental impact and meeting evolving consumer preferences. South Korea is well-positioned to shape the global edible insect market, driving innovation in sustainable protein production and securing a resilient future for global food systems.

Key words: Automation, AI, Circular economy, Sustainability, Food security.

INTRODUCTION

Korea has a long tradition of using insects for both food and medicinal purposes, as reflected in *Dong Eui Bo Gam*, the 1613 medical compendium by Heo Jun that records various traditional medicinal materials, including insects (Pemberton 1999; Yoshida 2012; Jeong *et al*. 2020). Rice grasshopper, *Oxya chinensis* has long been a seasonal delicacy. Silkworms (*Bombyx mori*) pupae, locally known as *beondaegi* are another example. The practice of rearing silkworms for silk and food in Korea dates back over 2,000 years and reflects a deep-rooted cultural heritage that is still preserved today. Silkworm larvae, pupae, and silk-fibroin hydrolysates are registered as food raw materials, while silkworm feces and *Bombyx*

Received: October 16, 2025; Accepted: December 29, 2025.
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batryticatus (Baekgangjam) are recognized as traditional Korean medicines. Among sericulture products, health-functional ingredients include silk-protein acid hydrolysates for immunity; fibroin hydrolysates for memory improvement; freeze-dried 5^{th} instar silkworm powder for blood sugar control; HongJam, made by steaming and freeze-drying mature silkworms, has been reported to offer health benefits such as preventing Alzheimer's and Parkinson's diseases, improving gastrointestinal functions, skin whitening, hair growth, and promoting a longer healthy life (Kim & Koh 2022). Beyond silkworms, crickets have also gained attention in modern Korea. While cricket farming is a newer industry compared to silkworms, it has rapidly evolved due to growing consumer demand. Crickets, such as *Teleogryllus emma* and *Gryllus bimaculatus*, are cultivated not only for human consumption but also for animal feed. The rise of cricket farming highlights Korea's ability to blend tradition with innovation by commercializing insects for broader markets. What sets Korea apart is its ability to integrate traditional practices with modern technologies (Meyer-Rochow *et al.* 2018). For example, while crickets were not historically consumed on a large scale, the country has adapted to emerging trends in insect farming, making them commercially viable. This innovation is seen as part of Korea's broader push to explore sustainable food sources in the face of global food security challenges. The combination of a cultural history of entomophagy and contemporary technological advances positions Korea as a leader in the global edible insect market.

Korea has become a notable player in the global edible insect industry in recent years (Caparros Megido *et al.* 2024). This rise is driven by increasing consumer demand for sustainable protein sources, government support for innovative agriculture, and a growing awareness of the health and environmental benefits of insects as food. Edible insect farming has seen substantial growth in the country, focusing on a variety of species, enhanced production techniques, and expanding international markets.

CURRENT SITUATION OF EDIBLE INSECT FARMING

As of 2024, Korea's edible insect farming industry is evolving rapidly, with significant developments in the commercial production of insects for human consumption, animal feed, and functional food products. The government, through the Ministry of Agriculture, Food, and Rural Affairs (MAFRA), has been actively promoting the industry as part of its strategy to address food security, rural development, and environmental concerns. According to the Major Statistics of the Ministry of Agriculture, Food and Rural Affairs (MAFRA 2025), 2,949 insect farms were operating in Korea in 2024. Table 1 presents the distribution of these farms across the country's provinces. The facilities used for insect farming include panel houses (1,012), vinyl houses (620), sunlight greenhouses (486), steel-frame concrete structures (467), containers (205), and other types of facilities (236). These facilities engage in the cultivation of various insect species, with modern production techniques being developed to ensure the quality and safety of edible insect products.

Insect species currently being farmed

In Korea, several insect species are being commercially farmed for food and feed purposes. These species include:

Table 1. Number of insect farms across provinces in Korea (MAFRA 2025).

Province	No. of farms
Gyeonggi (Seoul/Incheon)	749
Gangwon	122
Chungbuk	293
Chungnam (Daejeon/Sejong)	387
Jeonbuk	293
Jeonnam (Gwangju)	202
Gyeongbuk (Daegu)	508
Gyeongnam (Busan/Ulsan)	345
Jeju	50

1. Mealworms (*Tenebrio molitor*)

One of the most popular insect species in Korea, mealworms are farmed for both human consumption and animal feed due to their high protein content, favorable fatty acid profile, and potential use in various food products such as protein powders, snacks, and baked goods (Ghosh *et al*. 2017).

2. Crickets (*Gryllus bimaculatus*)

Crickets are another widely farmed species, known for their protein density, ease of farming, and sustainable production (Ghosh *et al*. 2017; Meyer-Rochow *et al*. 2021). Cricket-based products, such as powders, snacks, and even supplements, are gaining popularity in both the domestic and international markets.

3. Silkworm pupae (*Bombyx mori*)

Traditionally consumed in Korea as a street food called "beondegi," silkworm pupae are a rich source of protein and fiber. They are now being processed into modern food products, such as powders and functional ingredients. *Bombyx batryticatus* is registered as a Korean traditional medicine.

The production of silkworm by-products in Korea showed variable trends from 2018 to 2024, as illustrated in Fig. 1 (MAFRA 2025). Fresh silkworms recorded the highest production throughout the period, increasing from 110,256 kg in 2018 to a peak of 140,146 kg in 2020, and then slightly declining to 119,697 kg in 2024. In contrast, dried silkworm production decreased overall from 46,305 kg in 2018 to 27,194 kg in 2024, despite some fluctuations. The production of silkworm cocoons gradually declined from 15,179 kg in 2018 to 10,082 kg in 2024. Similarly, male pupae and *Cordyceps* showed a continuous reduction over the years, reaching 161 kg each in 2024. In contrast, HongJam (steamed and freeze-dried silkworm) production increased sharply from 196 kg in 2018 to 10,205 kg in 2020, before stabilizing at around 4,000 kg between 2021 and 2024. These trends indicate shifts in the production and utilization of different silkworm by-products over time.

4. Black soldier fly (*Hermetia illucens*)

Primarily farmed for animal feed, the black soldier fly larvae (BSFL) are also recognized for their role in waste recycling and as a protein source in aquaculture feed.

5. Rhinoceros beetle (*Allomyrina dichotoma*) and white-spotted flower chafer (*Protaetia brevitarsis*)

The larvae of these beetles are not only valued for their nutritional content but are also deeply rooted in traditional medicinal practices (Pemberton 1999; Ghosh *et al*. 2017).

6. Honey bee drone (*Apis mellifera*)

Honey bee drones were registered relatively recently, owing to their high nutritional value. With their rich protein content, essential amino acids, and abundant minerals, drone brood has the potential to serve as a valuable ingredient in both food and animal feed (Kim & Jung 2013; Ghosh *et al*. 2021).

These farms, however, tend to operate on a smaller-to-medium scale, focusing on specialized production for both food and therapeutic applications, showcasing the unique blend of cultural heritage and practical innovation in Korean insect farming.

The annual sales of insect products in Korea showed an overall increasing trend from 2020 to 2024, with species-wise sales distribution illustrated in Fig. 2 (MAFRA 2025). Total sales rose from 41,392 million KRW in 2020 to 52,818 million KRW in 2024. Among insect species, the white-spotted flower chafer recorded the highest sales, though it declined from 14,741 million KRW in 2020 to 11,282 million KRW in 2022, then recovered to 13,833 million KRW in 2024. Sales of mealworms increased steadily from 3,284 million KRW in 2020 to 6,822 million KRW in 2024, indicating growing market demand. Black soldier fly sales rose from 9,305 million KRW in 2020 to 11,589 million KRW in 2023, but slightly decreased to 10,140 million KRW in 2024. Meanwhile, rhinoceros beetle and stag beetle sales fluctuated moderately over the

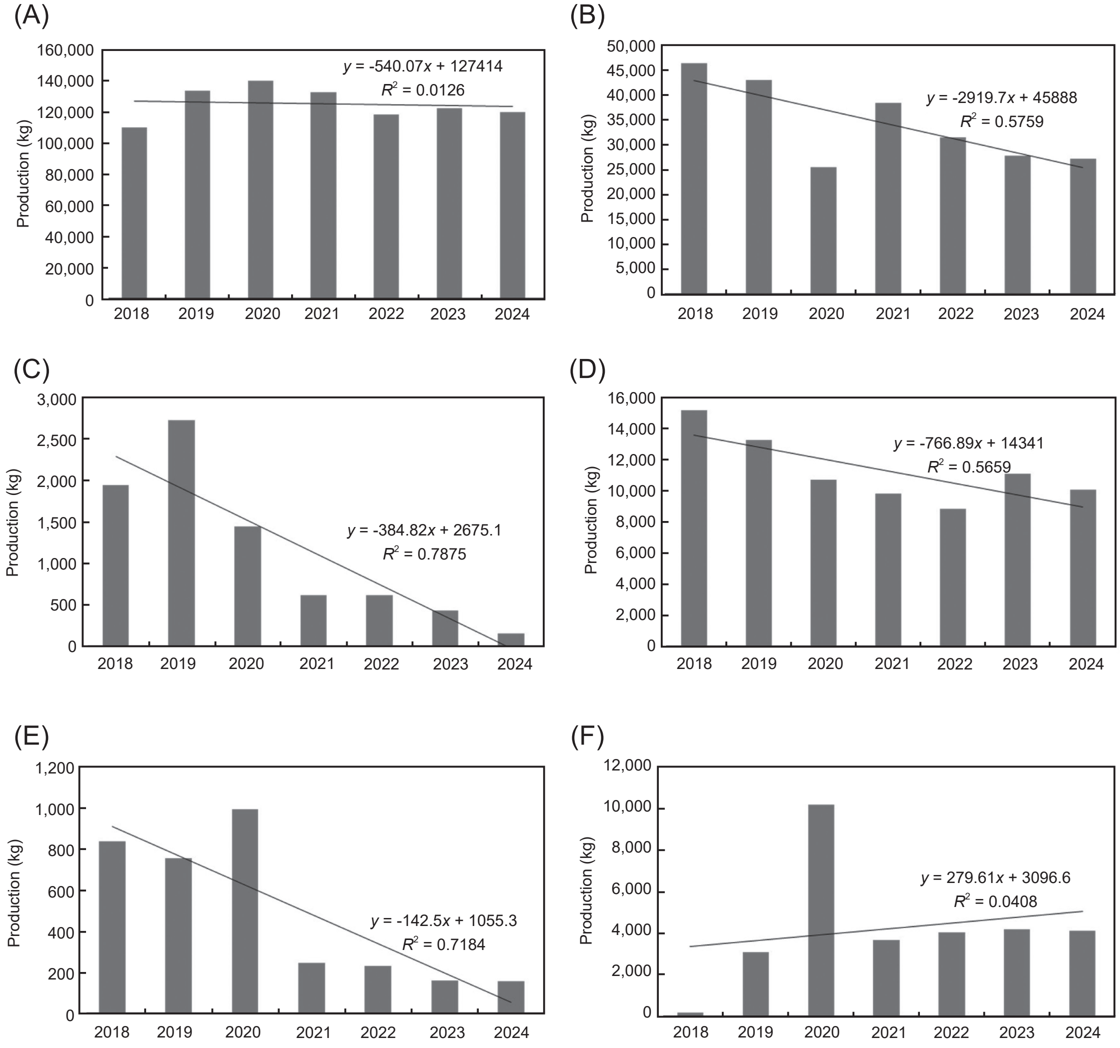

Fig. 1. Annual production trends of major silkworm by-products in Korea from 2018–2024 (kg). (A) Fresh silkworm; (B) dried silkworm; (C) male pupae; (D) silkworm cocoon; (E) *Cordyceps*; and (F) HongJam.

years. The cricket market also varied, peaking at 4,148 million KRW in 2023 before declining to 3,023 million KRW in 2024. In contrast, sales in the other insect category increased significantly from 6,492 million KRW in 2020 to 13,612 million KRW in 2024, reflecting diversification in the insect industry.

Large-scale production facilities

Several large-scale insect farming facilities have been established in Korea. These facilities are equipped with advanced technologies for mass breeding, processing, and packaging. Among the leading companies, Entomo Farms Korea and Bugs Korea are prominent examples, focusing on industrial-scale production. These large-scale facilities are integrated with automated systems for rearing, temperature control, feeding, and harvesting. This technological advancement not only ensures a higher yield but also meets stringent safety and quality standards that are crucial for both domestic and international markets. The farms can produce tons of insect biomass annually, which is further processed into

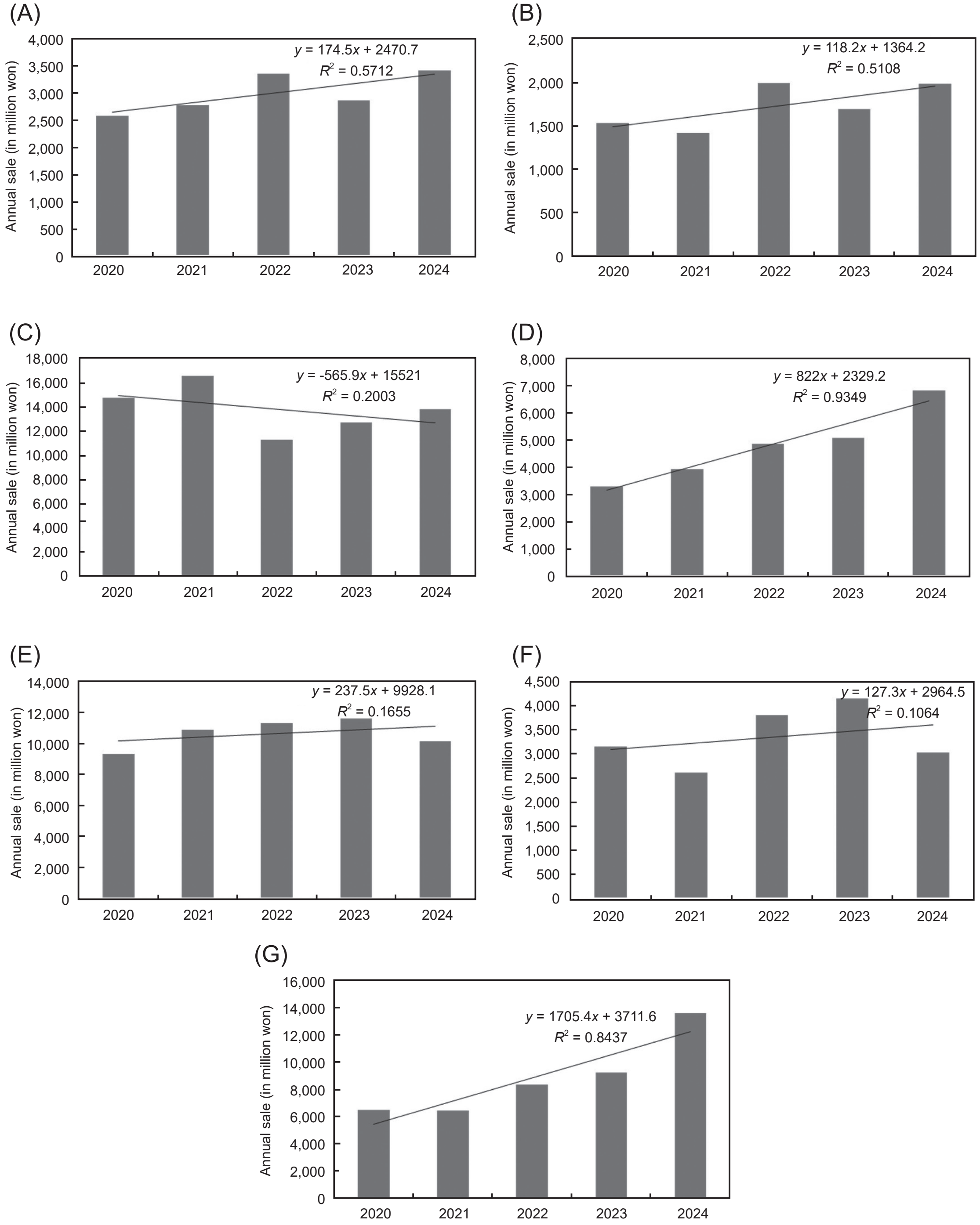

Fig. 2. Annual sales trends of major insect species in Korea from 2020–2024 (million KRW). (A) Rhinoceros beetle; (B) stag beetle; (C) white-spotted flower chafer; (D) mealworm; (E) black soldier fly; (F) cricket; and (G) others.

insect powders, protein bars, snacks, and other value-added products.

The Korean edible insect industry extends beyond insect production , encompassing various interconnected sectors designed to address specific industry requirements. Key categories include hardware, software, and services. Hardware refers to the physical devices and components essential for building edible insect production and product manufacturing facilities. Software solutions focus on applications that improve data management, automation, and analytics, enabling smarter decision-making and enhancing operational efficiency. The services segment provides consulting, implementation, and maintenance, ensuring the efficient deployment and utilization of edible insect feed technologies. Together, these components play a crucial role in driving market growth as businesses adopt integrated solutions that combine hardware, software, and services to meet their goals and remain competitive in a rapidly evolving landscape.

REGULATORY FRAMEWORK, GLOBAL PARTNERSHIPS, AND EXPANDING MARKET POTENTIAL FOR EDIBLE INSECTS IN SOUTH KOREA

Regulation and legislation

The Korean government is one of the earlier adapters of the global trend of the insect industry, making the registration of the act of Fosterage and Support of Insect Industry (2010, www.law.go.kr, hereafter, insect industry law). The act aims to promote the insect industry by providing a legal and institutional framework to support the breeding, production, processing, distribution, and utilization of insects. The law intends to enhance agricultural income, stimulate rural economies, and contribute to public well-being and environmental sustainability. Similarly, there are two more registrations regarding the traditional insect industry which are Functional Silviculture Promotion Act (2009), and Apiculture Promotion Act (2019). After establishing the Insect Industry Act, the Korean government has implemented several policies and regulatory frameworks to support the edible insect industry. In 2016, the Korean Food and Drug Administration (KFDA) approved the sale of certain edible insects for human consumption. This included species like mealworms and crickets, provided they meet safety standards for food hygiene (Table 2). The Insect Industry Promotion Act, established in 2011, serves as the primary legal framework governing insect farming in the country. It provides guidelines on the farming, processing, and distribution of insects for human consumption and animal feed. The act also outlines safety standards, labeling requirements, and permissible insect species for commercial use. In 2019, Korea further enhanced its regulatory infrastructure by introducing the Hazard Analysis and Critical Control Point (HACCP) certification for edible insect farms, ensuring that production facilities adhere to international food safety standards. Additionally, the government offers financial support to farmers venturing into the edible insect industry, including subsidies for research, facility upgrades, and market development.

International business collaborations

Korea is actively collaborating with international partners to expand its edible insect industry. Collaborations have been established with countries like the Netherlands, Thailand, and the United States to exchange expertise in insect farming technologies, research, and marketing strategies. Korean companies have been exploring partnerships to export their insect-based products to global markets, especially in Europe and North America, where demand for sustainable protein alternatives is increasing. The European Food Safety Authority (EFSA), through Regulation (EU) 2021/405, has approved certain insect species for consumption, thereby opening the door for Korean exporters to enter the European market (Spatola *et al*. 2024).

Research collaborations between Korean universities and international institutions are

Table 2. Edible insect species registered across different time periods in Korea.

Year	Registered insects for food Common name	Scientific name[z]
2014	Grasshoppers	*Oxya chinensis*
	Silkworm pupae	*Bombyx mori*
	Dried larva of *Bombyx mori* (4–5 instars; Baekgangjam) infected by *Beauveria bassiana*-*Bombyx batryticatus*	*Bombyx batryticatus*
2016	Mealworm larvae	*Tenebrio molitor*
	White spotted flower chafer larvae	*Protaetia brevitarsis*
	Rhinoceros beetle larvae	*Allomyrina dichotoma*
	Two-spotted cricket	*Gryllus bimaculatus*
2020	Super mealworm larvae (non-fat powder)[y]	*Zophobas morio*
2020	Honeybee drone pupae	*Apis mellifera*

[z] Scientific names were assigned based on the common names listed in the policy book published by the Rural Development Administration, Korea.
[y] Temporary registration.

also on the rise. These partnerships focus on the nutritional analysis of edible insects, the development of new processing techniques, and the exploration of insects' potential to address global food security challenges.

Potential market size

The edible insect market in Korea is projected to grow significantly in the coming years. According to a market research report by Future Market Insight, insect feed sales in Korea are expected to grow at an impressive compound annual growth rate (CAGR) of 23.6% through 2033. The market is projected to rise from USD 18.4 million in 2023 to USD 152.9 million by 2033 (https://www.futuremarketinsights.com/reports/insect-feed-industry-analysis-in-korea). Key drivers of market expansion include:

1. Growing consumer awareness

Increasing awareness of the environmental benefits of insect farming (lower greenhouse gas emissions, reduced water usage) and the high nutritional value of insects (rich in protein, vitamins, and minerals) is driving consumer interest.

2. Government support

Financial and regulatory support from the Korean government is accelerating the growth of the industry, encouraging more farmers to enter the market and increasing production capacity.

3. Innovative products

Korean companies are developing a wide range of insect-based products, including protein bars, insect powders, snacks, and even insect-infused beverages. These products are being marketed as healthy, sustainable, and innovative food solutions for modern consumers.

4. Export opportunities

With international markets like Europe and North America showing interest in edible insect products, Korea has the potential to become a leading exporter of high-quality, safe, and innovative insect-based foods.

CONTEMPORARY ISSUES OF THE EDIBLE INSECT INDUSTRY

The role of automation and artificial intelligence (AI) in insect farming: Revolutionizing large-scale production

The rapid expansion of edible insect farming demands innovative solutions to address challenges in scalability, efficiency, and precision. Automa-

tion, integrated with AI, is playing a crucial role in transforming insect farms into highly efficient production facilities, as depicted in Fig. 3. The combination of AI and automation technologies enables faster, more accurate, and large-scale insect production, driving sustainability while ensuring profitability.

1. The potential role of automation in insect farming

Automation in insect farming uses advanced machinery, robotics, and sensors to handle various tasks such as insect breeding, feeding, harvesting, and monitoring environmental conditions. It significantly reduces human intervention, streamlines operations, and improves the overall quality and consistency of production.

Some of the critical areas where automation can revolutionize insect farming include:

1.1. Automated rearing systems: AI-driven machinery can be used to automate the process of rearing insects, from egg-laying to larval development. Machines equipped with precise temperature and humidity controls ensure optimal conditions for insect growth, reducing mortality and improving development speed.

1.2. Feeding automation: Ensuring that insects receive a balanced and consistent diet is critical to maximizing growth rates and nutritional content. Automated feeding systems use sensors to detect insects' feeding status and deliver precise amounts of food, reducing waste and optimizing resource use.

1.3. Harvesting automation: Harvesting insects at the correct time is crucial for achieving high yields and ensuring the desired nutritional content. Automated systems, guided by AI algorithms, can detect the optimal harvest time based on factors like the size and weight of the insects. These systems can efficiently collect, sort, and process large volumes of insects with minimal labor.

1.4. Environmental monitoring and control: AI-driven sensors monitor the insect farm's environmental parameters, such as temperature, humidity, and ventilation,

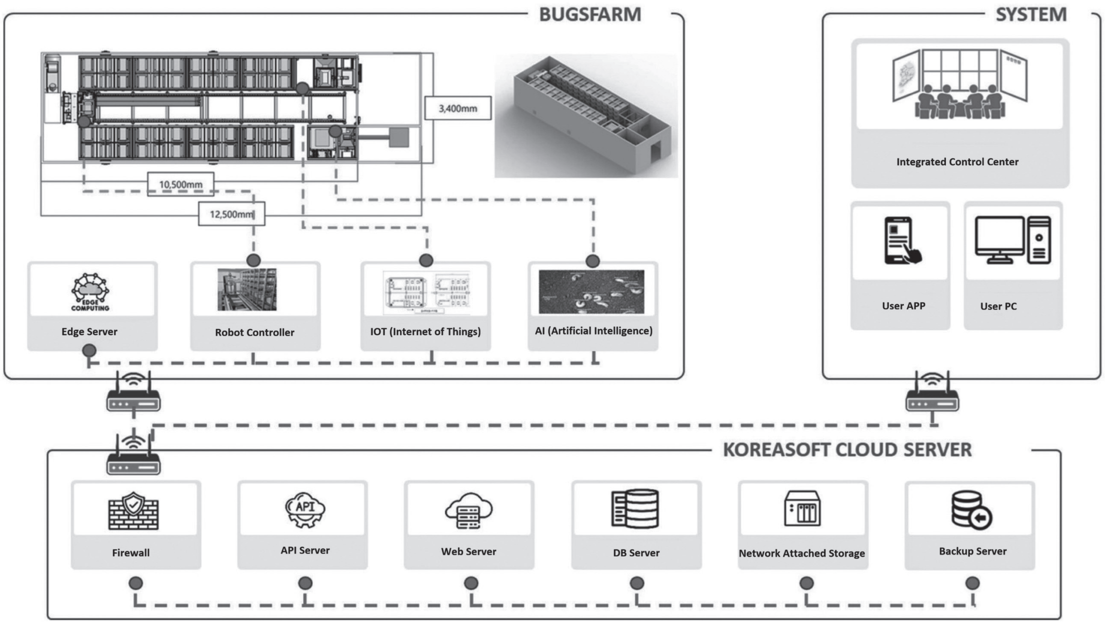

Fig. 3. Overview of an automated insect production facility (photo credit: Korea Soft).

ensuring optimal conditions for growth. Real-time data collection and analysis enable immediate adjustments in response to changes, helping to prevent disease outbreaks and improving overall insect health.

1.5. **Waste management:** Insect farms produce organic waste, and AI-powered automation can help manage and recycle waste materials. Black soldier fly larvae, for instance, are highly efficient at converting organic waste into protein, and automated waste processing systems can integrate this process, turning waste into a valuable resource.

2. The practical role of artificial intelligence in the improvement of insect farming

AI is increasingly being incorporated into farming operations to optimize production, improve decision-making, and reduce costs (Fig. 4). In insect farming, AI can enhance efficiency across various stages of the production process by leveraging machine learning, data analytics, and computer vision.

2.1. **Data-driven insights and predictive analytics:** AI algorithms analyze vast amounts of data collected from automated systems, providing valuable insights into insect growth patterns, feed efficiency, and environmental factors, as we discussed earlier. Predictive models can forecast growth cycles, allowing farmers to plan and optimize production schedules, anticipate challenges, and adjust strategies accordingly.

2.2. **Computer vision for monitoring and quality control:** Computer vision technologies powered by AI can visually monitor insect development and health. These systems can detect irregularities, such as diseases, malformations, or undernourished larvae, enabling early intervention. They can also assess the quality of harvested insects, ensuring that only those meeting specific criteria are processed for human consumption or animal feed.

2.3. **AI-driven genetic optimization:** AI can aid in selective breeding programs aimed at optimizing insect strains for faster growth, higher protein content, or disease resistance. By analyzing genetic data, AI algorithms can identify desirable traits and guide breeding decisions, leading to more efficient insect production over time.

2.4. **Supply chain optimization:** AI helps optimize the logistics of insect production by streamlining supply chain operations, from sourcing raw materials (like feed) to distributing final products. Algorithms can predict demand fluctuations, reducing overproduction or shortages and improving the overall profitability of insect farming operations.

Korea is at the forefront of agricultural

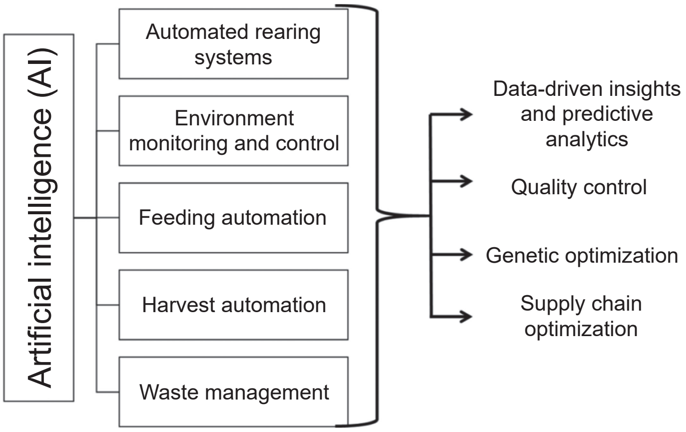

Fig. 4. Role of artificial intelligence (AI) in automated insect production facility.

technological innovation, including insect farming. The country has been investing heavily in smart farming technologies, including AI, to improve food production efficiency and address issues related to labor shortages, food security, and sustainability. Although the integration of AI in insect farming is still in its early stages, several promising developments are emerging in Korea and globally. Korea's insect farming industry is starting to adopt AI-driven technologies in a few large-scale operations. Companies like Bugs Korea and Entomo Farms Korea are exploring automated systems to manage temperature, humidity, and feeding schedules. Additionally, partnerships with tech firms and research institutions are being formed to develop AI applications tailored for insect farming. The Korean Rural Development Administration (RDA) has been actively promoting research into smart farming, and insect farms are included in these initiatives. The RDA is working with agricultural tech companies to develop automated insect farming systems that can be managed remotely via AI-powered apps, giving farmers real-time updates and control over their production.

Balancing sustainability and large-scale edible insect farming: A path forward

Edible insects have emerged as a promising solution to address the world's growing demand for sustainable food sources. Two primary pillars support the promotion of edible insect consumption and production: nutritional benefits and sustainability (van Huis *et al.* 2013). Insects are highly nutritious, boasting dense protein, essential minerals, and primarily unsaturated fats beneficial for cardiovascular health. In terms of sustainability, insects require minimal natural inputs, including land, water, and feed, while also emitting negligible amounts of greenhouse gases (GHGs) such as methane, unlike conventional livestock farming (e.g., cattle, swine, and poultry). However, as insect farming scales up to meet increasing global demand, a new challenge arises- the carbon footprint of large-scale automated or semi-automated insect production. Large production facilities, particularly those equipped with automated systems, require substantial energy for environmental control, such as heating, ventilation, air conditioning (HVAC), automated feeding, and processing machinery. As a result, this intensification could elevate the carbon footprint, potentially undermining the sustainability advantage of small-scale insect farms. To address this challenge, we must explore ways to ensure sustainability even with the growing shift toward large-scale insect production.

1. Small vs. large-scale insect farming: Carbon footprint comparison

1.1. Small and medium-scale producers: In regions like Southeast Asia, small and medium-scale insect farming is naturally supported by favorable climatic conditions (Hanboonsong *et al.* 2013; Ghosh *et al.* 2025). Many insect species, such as crickets, mealworms, and black soldier flies, thrive in warm, humid environments, which align closely with the natural conditions of tropical and subtropical areas. In these regions, insect farmers often rely on ambient environmental conditions to rear insects, without needing extensive energy inputs to regulate temperature or humidity. These small-scale farms typically have a minimal carbon footprint due to low energy consumption, limited reliance on artificial environmental controls, reduced transportation requirements, as production often occurs close to local markets (Barragán-Fonseca *et al.* 2025; Ghosh *et al.* 2025).

1.2. Large-scale, automated insect production: As the industry grows and large-scale production facilities are established, particularly in regions with less favorable climates, automation and mechanization introduce energy-intensive processes. This includes: Heating and cooling systems to maintain optimal temperatures for insect growth in colder climates; humidity control- essential for preventing desiccation or exces-sive

moisture, both of which can affect insect health and growth; automated feeding and harvesting- robots and machines used for these tasks are energy-intensive, especially in facilities producing large volumes of insects. While automation brings the benefits of efficiency, scalability, and consistency, it also introduces higher energy demands, contributing to a greater carbon footprint, especially if the energy used comes from non-renewable sources.

2. Strategies to ensure sustainability in large-scale insect farming

To maintain the environmental benefits of insect farming while scaling up, it is crucial to adopt energy-efficient practices, renewable energy sources, and innovative technological solutions. One of the most effective ways to offset the energy demands of large-scale insect farming is to integrate renewable energy sources such as solar, wind, or geothermal power. Insect farming facilities can utilize renewable energy to power automated systems, environmental controls, and processing machinery. This not only reduces reliance on fossil fuels but also aligns with broader global efforts to mitigate climate change. As automation becomes more integral to large-scale insect production, it is important to develop energy-efficient technologies that minimize power consumption without sacrificing productivity.

Adoption of circular economy practices

The concept of a circular economy is particularly well-suited to insect farming. Insect farms can be designed to recycle waste products, both within the facility and from external sources. For instance:

1. Organic waste recycling

Insects like black soldier fly larvae are highly efficient at converting organic waste (e.g., food scraps, agricultural by-products) into valuable biomass (Bruno *et al*. 2025). By incorporating organic waste streams from other industries into their feed, insect farms can reduce the need for specially cultivated feedstock, thereby minimizing land use and water consumption associated with feed production.

2. Waste-to-energy systems

Facilities can use anaerobic digestion or composting to convert waste by-products (such as insect frass or exoskeletons) into biogas, which can then be used as an energy source within the farm (Odoi-Yorke *et al*. 2025).

Product development and value addition

Promoting edible insects in communities familiar with entomophagy can be achieved by establishing sustainable, scientifically sound rearing systems. Such systems ensure a consistent supply of nutritious products while enhancing food safety. In contrast, harvesting edible insects from the wild poses significant risks to biodiversity and raises safety concerns about consuming wild-caught species. For communities unfamiliar with edible insects, characterized by negative perceptions towards entomophagy, it is essential to shift the focus from whole insects to their incorporation as ingredients in food products. For example, insect flour can be blended with wheat flour to produce bakery items, while insect protein hydrolysate can serve as a valuable ingredient in protein supplements. This approach can facilitate greater consumer acceptance by integrating insects into familiar food forms. Furthermore, diversifying insect-based products offers an effective strategy for promoting their benefits. Using insects as animal feed can reduce demand for traditional feed sources, which often have significant environmental impacts. Insect protein sources for poultry and fish feed can be more sustainable alternatives to current animal feed practices.

The diversification of insect products not only enhances the nutritional profile of diets but also contributes to environmental sustainability. By presenting insects as a functional component of food rather than as standalone items, we can bridge the gap between the nutritional and ecological advantages of edible insects and

human consumption, ultimately benefiting both health and the environment.

CONCLUSIONS

The edible insect farming industry in Korea is on a promising trajectory, supported by government initiatives, innovative product development, and growing domestic and international demand. With a strong regulatory framework, increasing investment in large-scale production facilities, and a focus on sustainability, Korea is poised to become a global leader in edible insect farming. As consumer acceptance grows and technological advancements continue, the industry holds significant potential for contributing to both food security and environmental sustainability in the coming decades.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge Korea Soft for kindly providing the flowchart illustrating the overview of an automated insect production facility, and National Research Foundation of Korea (NRF-2018R1A6A1A03024862).

REFERENCES

Barragán-Fonseca, K. B., D. Gómez, C. H. Lalander, D. Dzepe, and S. Y. Chia. 2025. Review- Insect farming for food and feed in the Global South: Focus on black soldier fly production. Animal 19:101397. doi:10.1016/j.animal.2024.101397

Bruno, D., M. Orlando, E. Testa, M. C. Miino, G. Pesaro, M. Miceli, … G. Tettamanti. 2025. Valorization of organic waste through black soldier fly: On the way of a real circular bioeconomy process. Waste Manag. 191:123–134. doi:10.1016/j.wasman.2024.10.030

Caparros Megido, R., F. Francis, E. Haubruge, P. Le Gall, J. K. Tomberlin, C. D. Miranda, … A. van Huis. 2024. A worldwide overview of the status and prospects of edible insect production. Entomol. Gen. 44:3–27. doi:10.1127/entomologia/2023/2279

Ghosh, S., B. Chuttong, K. Danmek, P. Aedtem, V. B. Meyer-Rochow, and C. Jung. 2025. Sustainability analysis of insect farming; case study of farming sago palm weevil (*Rhynchophorus ferrugineus*) in south Thailand. J. Asia-Pac. Entomol. 28:102422. doi:10.1016/j.aspen.2025.102422

Ghosh, S., S. M. Lee, C. Jung, and V. B. Meyer-Rochow. 2017. Nutritional composition of five commercial edible insects in South Korea. J. Asia-Pac. Entomol. 20:686–694. doi:10.1016/j.aspen.2017.04.003

Ghosh, S., V. B. Meyer-Rochow, and C. Jung. 2021. Honey bees and their brood: A potentially valuable resource of food, worthy of greater appreciation and scientific attention. J. Ecol. Environ. 45:31. doi:10.1186/s41610-021-00212-y

Hanboonsong, Y., T. Jamjanya, and P. B. Durst. 2013. Six-legged Livestock: Edible Insect Farming, Collection and Marketing in Thailand. Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific. Bangkok, Thailand. 57 pp.

Jeong, D., N. Min, Y. Kim, S. R. Kim, and O. Kwon. 2020. The effects of feed materials on the nutrient composition of *Protaetia brevitarsis* larvae. Entomol. Res. 50:23–27. doi:10.1111/1748-5967.12404

Kim, H. S. and C. Jung. 2013. Nutritional characteristics of edible insects as potential food materials. Kor. J. Apic. 28:1–8.

Kim, K. Y. and Y. H. Koh. 2022. The past, present, and future of silkworm as a natural health food. Food Sci. Ind. 55:154–165. (in Korean with English abstract) doi:10.23093/FSI.2022.55.2.154

Meyer-Rochow, V. B., R. T. Gahukar, S. Ghosh, and C. Jung. 2021. Chemical composition, nutrient quality and acceptability of edible insects are affected by species, developmental stages, gender, diet, and processing method. Foods 10:1036. doi:10.3390/foods10051036

Meyer-Rochow, V. B., S. Ghosh, and C. Jung. 2018. Farming of insects for food and feed in South Korea: Tradition and innovation. Berl. Münch. Tierärztl. Wochenschr. 131:236–244. doi:10.2376/0005-9366-18056

Ministry of Agriculture, Food and Rural Affairs (MAFRA). 2025. Survey Report on the Current Status of the Insect Industry. Ministry of Agriculture, Food and Rural Affairs. Daejeon, Korea. 77 pp. (in Korean)

Odoi-Yorke, F., A. N. A. Abbey, E. Asante, K. K. Ansah, D. B. Asare, G. Mensah, … L. Atepor. 2025. Harnessing black soldier fly larvae for sustainable biofuel production: A review of global research trends and future directions. Intl. J. Energy Res. 2025:8032373. doi:10.1155/er/8032373

Pemberton, R. W. 1999. Insects and other arthropods used as drugs in Korean traditional medicine. J. Ethnopharmacol. 65:207–216. doi:10.1016/S0378-8741(98)00209-8

Spatola, G., A. Giusti, S. Mancini, L. Tinacci, R. Nuvoloni, F. Fratini, … A. Armani. 2024. Assessment of the

information to consumers on insects-based products (novel food) sold by e-commerce in the light of the EU legislation: When labelling compliance becomes a matter of accuracy. Food Control 162:110440. doi:10.1016/j.foodcont.2024.110440

van Huis, A., J. Van Itterbeeck, H. Klunder, E. Mertens, A. Halloran, G. Muir, and P. Vantomme. 2013. Edible Insects: Future Prospects for Food and Feed Security. Food and Agriculture Organization of the United Nations. Rome, Italy. 187 pp.

Yoshida, K. 2012. Philological and historical research on *Dong Eui Bo Gam* at the Nanjing University of Traditional Chinese Medicine. J. Med. Soc. Toho. 59:54–59.

韓國食用昆蟲產業之現況分析：現今議題、面臨挑戰與未來發展方向

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摘要

Ghosh, S., S. Sun, and C. Jung. 2026. Current status of edible insect industry in Korea: Contemporary issues, challenges, and path ahead. J. Taiwan Agric. Res. 75(2):171–184.

韓國食用昆蟲產業已從原先僅利用蠶蛹等昆蟲作為食品與藥物的根深蒂固的傳統，發展成為一個融合先進技術與永續發展的現代化產業。近年來，此產業得到顯著發展，約有 245 個運作中的昆蟲養殖場，養殖著麵包蟲、蟋蟀、蠶蛹、黑水虻幼蟲以及蜜蜂雄蜂等昆蟲。在 2011 年的《昆蟲產業促進法》與 Hazard Analysis and Critical Control Point (HACCP) 認證等監管架構的支持下，該產業旨在解決糧食安全、農村發展及環境問題。大規模生產設施與人工智慧驅動的自動化提高了生產效率，而全球合作與出口策略使韓國在全球食用昆蟲市場中居於領先地位。然而，為了擴大生產規模卻也帶來是否足以永續發展的挑戰，尤其是在能源使用與碳足跡方面。為維持環境效益，採用包括再生能源、實施循環經濟以及創新加工等策略至關重要。透過將昆蟲融入常見的食品與動物飼料中，可提高消費者的接受度。總體而言，韓國食用昆蟲產業融合了文化遺產與尖端科技，為糧食安全、永續發展及健康作出了具體貢獻。該行業的持續成長取決於如何在規模化生產與環境影響之間取得平衡，並滿足不斷變化的消費者偏好。韓國擁有塑造全球食用昆蟲市場的有利條件，能夠推動永續蛋白質生產的創新，並確保全球糧食體系擁有韌性的未來。

關鍵詞：自動化、人工智慧、循環經濟、永續性、糧食安全。

投稿日期：2025 年 10 月 16 日；接受日期：2025 年 12 月 29 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):185–199 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0002

Feature Article

Plant Disease Management Strategies for Sustainable Agriculture

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Abstract

Huang, H. C. 2026. Plant disease management strategies for sustainable agriculture. J. Taiwan Agric. Res. 75(2):185–199.

The outbreak and spread of crop diseases result from the interactions among plant pathogens, host plants, and environmental conditions. Improper cultural practices, such as the use of diseased seeds, susceptible varieties, and inappropriate cultivation practices, may lead to the emergence or resurgence of crop diseases. Crop disease management is crucial in modern agriculture. In this era of energy conservation and environmental protection, all crop disease control methods must be effective, economical, and environmentally friendly. Crop disease management methods such as the use of clean seeds, disease-resistant varieties, crop rotation, biological control, organic soil amendment, and cultural practices are considered viable alternatives to synthetic chemical pesticides. The prospects and applications of these environmentally friendly plant disease control methods are further discussed using fungal and bacterial diseases as examples.

Key words: Allelopathy, Biological control, Crop rotation, Disease resistance, Ecology, Epidemiology, Soil amendment, Insect-pathogen relationship.

INTRODUCTION

Plant disease outbreaks require sufficient numbers of virulent pathogens to initiate infection, susceptible hosts to promote disease development, and environmental conditions that favor further spread of the disease. Inappropriate farming practices in modern crop production often lead to the emergence and recurrence of crop diseases. For example, farmers in Alberta, Canada, use sprinkler irrigation to increase crop yields of alfalfa, beans, and sugar beets. This cultivation method has led to serious disease outbreaks in these crops, including Verticillium wilt of alfalfa (*Verticillium albo-atrum*) (Howard *et al*. 1991), white mold of beans (*Sclerotinia sclerotiorum*) (Huang *et al*. 1988), *Pythium* damping-off of sugar beets (*Pythium* spp.), and bacterial wilt of beans (*Curtobacter flaccumfaciens* pv. *flaccumfaciens*) (Hsieh *et al*. 2002). Other cultural practices, such as planting pathogen-infected seeds or susceptible crop varieties, can also lead to disease outbreaks, resulting in severe crop losses.

Verticillium wilt of alfalfa, caused by *V. albo-atrum*, was discovered in Sweden in 1918 and caused severe losses to alfalfa grown in cooler regions of Europe in the 1950s (Bergstrom 1984). In North America, the disease was first reported on alfalfa in the United States in 1977 (Graham *et al*. 1977) and in Canada in 1979 (Sheppard 1979). In 1994, Verticillium wilt of alfalfa was also reported in Hokkaido, Japan

Received: October 2, 2025; Accepted: January 28, 2026.
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(Sato 1994). Results from annual field surveys conducted in Alberta from 1980 to 1994 indicate that Verticillium wilt of alfalfa occurs primarily in irrigated alfalfa but is rarely seen in dryland alfalfa (Table 1). *S. sclerotiorum* is another fungal pathogen with a wide host range that is distributed worldwide (Boland & Hall 1994). In Canada, *S. sclerotiorum* causes disease in many commercial crops, including oilseed crops (sunflower, canola, and safflower), pulse crops (beans, peas, and lentils), and vegetable crops (lettuce, carrots) (Bardin & Huang 2001). This disease is serious in Alberta, especially in irrigated crops such as beans (Table 2). *Pythium* damping-off (*Pythium ultimum*) is an important seedling disease of vegetables and other crops in many countries. In Alberta, *Pythium* damping-off is important on safflower, canola, peas, and sugar beet (Huang *et al*. 1992a; Bardin *et al*. 2003). The purpose of this article is to explore effective and environmentally safe plant disease management strategies using these three high-profile fungal diseases as examples.

BIOLOGY AND EPIDEMIOLOGY OF PLANT PATHOGENS

Basic research to understand the biology and epidemiology of plant pathogens is critical to developing effective and rational crop disease management strategies. For example, the pathogen *S. sclerotiorum* produces black sclerotia that can survive extreme weather conditions. This explains why Sclerotinia disease of crops occurs worldwide, from cold temperate regions to the tropics. In addition, the black sclerotia of *S. sclerotiorum* can germinate myceliogenically to produce white mycelia or carpogenically to produce ascocarps, releasing large numbers of airborne ascospores. Therefore, different sclerotial germination patterns can lead to distinct diseases in plants. For example, the black sclerotia in the soil germinate myceliogenically to produce white mycelia, which either attack the roots of sunflower seedlings, causing damping-off disease, or attack the roots of older plants, resulting in

Table 2. Annual survey of Sclerotinia white mold of bean (*Sclerotinia sclerotiorum*) in Alberta, Canada (1988–2011).

	Diseased/surveyed (%)	
Year	Irrigated fields	Reference
1988	3/5 (60%)	Huang & Phillippe (1989)
1993	31/31 (100%)	Huang & Erickson (1994a)
1994	33/37 (89%)	Huang *et al*. (1995)
1995	18/18 (100%)	Huang *et al*. (1996)
1998	20/21 (95%)	Huang & Erickson (1999)
1999	22/22 (100%)	Huang & Erickson (2000)
2007	27/28 (96%)	Erickson & Balasubramanian (2008)
2009	41/43 (95%)	Erickson & Balasubramanian (2010)
2011	27/30 (90%)	Erickson & Chatterton (2012)

Table 1. Annual survey of Verticillium wilt of alfalfa (*Verticillium albo-atrum*) in Alberta, Canada (1980–1994).

	Diseased/surveyed (%)		
Year	Irrigated fields	Dryland fields	Reference
1980	5/49 (10%)	0/24 (0%)	Howard *et al*. (1991)
1981	22/86 (26%)	0/28 (0%)	Howard *et al*. (1991)
1982	71/791 (9%)	1/247 (0.4%)	Howard *et al*. (1991)
1983	12/56 (21%)	0/24 (0%)	Howard *et al*. (1991)
1984	10/54 (19%)	0/32 (0%)	Howard *et al*. (1991)
1985	16/35 (46%)	2/18 (11%)	Howard *et al*. (1991)
1986	17/65 (26%)	0/28 (0%)	Howard *et al*. (1991)
1993	7/10 (70%)	(No data)	Huang & Erickson (1994b)
1994	18/18 (100%)	(No data)	Huang & Erickson (1995)

wilt of sunflower plants (Dorrell & Huang 1978; Huang & Dueck 1980). On the contrary, black sclerotia in the soil germinate carpogenically to produce ascocarps and release a large number of airborne ascospores, which attack the aboveground tissues of plants and cause diseases such as sunflower head rot, safflower head rot, pea pod rot (Huang & Kokko 1992) and bean white mold. Therefore, understanding the germination behavior of sclerotia of *S. sclerotiorum* is crucial to adopt the correct strategies to control Sclerotinia diseases in crops. Taking the biocontrol of Sclerotinia diseases by *Coniothyrium minitans* as an example, to effectively control Sclerotinia wilt of sunflower, *C. minitans* should be applied via soil treatment (Huang 1980; McLaren *et al*. 1994). However, for effective control of bean white mold, *C. minitans* should be applied as an air-spray (Huang *et al*. 2000) or as a soil treatment before sowing and air-spray during the flowering period (Huang & Erickson 2004a).

Verticillium wilt of alfalfa caused by *V. albo-atrum* is a good example of the versatility of plant pathogens. This pathogen has a narrow host range but can be transmitted through multiple routes (Huang 2003). Since Verticillium wilt is a systemic disease of alfalfa, the pathogen is distributed in the vascular system of infected roots, stems, leaves, pods, and seeds (Huang *et al*. 1985; Huang 1989). Therefore, Verticillium wilt of alfalfa is an important quarantine disease in many countries, including China and Japan. Exports of alfalfa hay or seeds to these countries are strictly controlled by quarantine rules. In addition, under humid conditions in the field, large numbers of *V. albo-atrum* spores (conidia) are produced on old diseased alfalfa plants. These spores can be spread by running water, agricultural machinery, or insect pests such as aphids (Huang *et al*. 1983), grasshoppers (Huang & Harper 1985) and alfalfa weevils (Harper & Huang 1984). These insects are important alfalfa pests in Canadian prairie and are effective vectors of Verticillium wilt of alfalfa (Huang 2003).

The alfalfa leafcutter bee (*Megachile rotundata*) is an important pollinating insect for alfalfa seed production. A field study showed that many leafcutter bees were contaminated with *V. albo-atrum* spores while foraging in diseased alfalfa fields (Huang *et al*. 1986b). Leafcutter bees in the fields also cut leaf fragments infected with *V. albo-atrum* and use them to make cocoons in their hives (Huang & Richards 1983). Contamination of leafcutter bee cocoons with *V. albo-atrum* may pose potential problems for international trade in this pollinating insect. Further studies conducted indoors (Huang & Kokko 1985) and in the field (Huang *et al*. 1986b) showed that alfalfa pollen grains are susceptible to infection by *V. albo-atrum*. Since alfalfa leafcutter bees are important pollinators for alfalfa seed production, infection of alfalfa pollen by *V. albo-atrum* may further affect alfalfa seed production. Therefore, in addition to insect pests, this pollinator may also be a potential vector when foraging in alfalfa seed fields where Verticillium wilt occurs.

Another study also suggested that Verticillium wilt of alfalfa may be transmitted by ruminants (e.g., lambs) grazing on diseased alfalfa pastures (Huang *et al*. 1986a). All these basic studies on the survival, biological behavior, and epidemiology of *S. sclerotiorum* and *V. albo-atrum* will provide the basis for developing effective strategies for the sustainable management of these two important fungal diseases.

STRATEGIES FOR SUSTAINABLE MANAGEMENT OF CROP DISEASES

To minimize the over-reliance on chemical pesticides, some conventional measures and new options for sustainable crop disease management are discussed below.

Phytosanitary measures

Phytosanitary measures are used to prevent the spread of plant diseases between countries. For example, Verticillium wilt of alfalfa (*V. albo-atrum*) is listed as a quarantine disease in China.

All alfalfa hay shipped to China from the USA (Anonymous 2012) or Canada (Anonymous 2021) must be certified Verticillium wilt-free. Bacterial wilt of bean caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* is an important seedborne disease in North America, South America, Europe, and Australia (Huang *et al*. 2009) and is a quarantine disease in many Asian countries, including China (Chun Li, Personal Communication). Four variants of the bacterial wilt pathogen in beans have been reported: yellow, orange, purple, and pink variants (Huang *et al*. 2009). The European and Mediterranean Plant Protection Organization (EPPO) imposes quarantine regulations to prevent the import of bean seeds infected with the bacterial wilt pathogen (OEPP/EPPO 1982). Therefore, phytosanitary measures are a reasonable measure to prevent the spread of plant diseases through commercial trade of seeds or other planting materials such as seedlings or tubers.

The development of rapid detection methods for plant pathogens is critical for the implementation of phytosanitary regulations. Taking bacterial wilt of beans as an example, Tegli *et al*. (2002) developed a rapid, sensitive, and specific PCR-based assay for the detection of *C. flaccumfaciens* pv. *flaccumfaciens* (Cff) in infected bean seeds. McDonald & Wong (2000) developed a cocktail of antibodies to screen commercial bean seeds for the presence of various variants (yellow, orange, purple, or pink variants) of the bacterial pathogen. Another example is Verticillium wilt of alfalfa, for which Christen (1982) developed a selective medium specifically for the detection of the pathogen *V. albo-atrum* in soil, infected alfalfa seeds, and infected alfalfa hay. These two examples demonstrate that the development of rapid detection methods for plant diseases varies by disease, and therefore, focus should be placed on the effectiveness and efficiency of each detection method.

Use pathogen-free seeds or seedlings for planting

Seeds, tubers, or other planting materials infected with plant pathogens have the potential to introduce these diseases into the field. Seed-borne diseases are common in cultivated crops. For example, *V. albo-atrum* (*Vaa*) is a seed-borne pathogen of alfalfa (Sheppard & Needham 1980; Huang *et al*. 1985), and the use of *Vaa*-infected alfalfa seeds may lead to outbreaks of Verticillium wilt. *Erwinia rhapontici* is another seed-borne pathogen that causes pink seed disease in many crops, including peas, beans, lentils, chickpeas, and durum wheat (Huang *et al*. 2003a, 2003b; Hsieh *et al*. 2010). Field studies have shown that planting *E. rhapontici*-infected pea seeds reduces pea emergence, seedling vigor, and seed yield (Huang & Erickson 2004c). The pink seed pathogen survives well under the cold winter conditions of the Canadian prairies (Hsieh *et al*. 2010). *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* is another seed-borne pathogen that causes bacterial wilt of beans in many countries (Huang *et al*. 2009). All these examples suggest that seed-borne pathogens may be the primary source of inoculum for many crop diseases. Therefore, using pathogen-free seeds or seedlings for planting is a prerequisite for preventing crop diseases. It is important to use isolated fields with good field sanitation to produce healthy seeds for planting.

Breeding crops for disease resistance

Plant disease outbreaks are often the result of growing commercial varieties that are susceptible to pathogens. Breeding disease-resistant crops is the most effective and environmentally friendly way to protect crops from plant diseases. For example, Verticillium wilt of alfalfa was discovered in Canada in 1979 (Sheppard 1979), but due to the lack of resistant commercial varieties, it quickly became a major disease spreading across the country. In an effort to find a solution to Verticillium wilt of alfalfa, Agriculture and Agri-Food Canada established the National Alfalfa Breeding Program in 1981, with the specific goal of developing new alfalfa varieties resistant to this disease. This breeding effort resulted in the release of three new alfalfa varieties, ‘Barrier’ (Hanna & Huang 1987), ‘AC Blue J’ (Acharya *et al*. 1995) and ‘AC Longview’ (Acharya &

Huang 2000), that are high-yielding and resistant to Verticillium wilt (Huang *et al*. 1994). The economic benefits of growing these Verticillium wilt-resistant alfalfa varieties in Western Canada are estimated to be CDN$26.6 million per year (Smith *et al*. 1995).

Developing rapid and effective techniques to screen crop varieties or lines for disease resistance is a critical step in ensuring the success of crop breeding programs. For example, Fusarium wilt, caused by *F. oxysporum* f. sp. *chrysanthemi*, is an important disease of cold-tolerant chrysanthemums in Manitoba, Canada (Huang *et al*. 1992b). An indoor screening using chrysanthemum stem cuttings can effectively identify chrysanthemum varieties or lines that are resistant to Fusarium wilt (Huang *et al*. 1993). Another example is the release of three alfalfa varieties resistant to Verticillium wilt (Acharya & Huang 2003), which was attributed to the use of an effective indoor screening technique for this disease (Huang & Hanna 1991). In addition to conventional screening methods for crops disease resistance, other approaches, such as molecular methods, should be explored to accelerate plant breeding programs. At the same time, collaboration among researchers such as plant pathologists, plant breeders, and molecular biologists is crucial to ensure the success of crop breeding programs.

Soil amendment

Some plant species may contain allelopathic chemicals that are harmful to plant pathogens (Patrick 1986). Soil amendment with allelopathic plant tissues or other agricultural wastes (e.g., manure, compost) may help control crop diseases (Huang & Huang 1993). For instance, amending soil with 3% (w/w) lentil, canola, or sweet clover straw not only inhibited the production of apothecia of *S. sclerotiorum* but also promoted the growth of soil microflora (Huang *et al*. 2002a; Huang & Chou 2005). In addition to controlling plant pathogens, Moyer & Huang (1997) reported that 1% aqueous extracts of lentil straw, oat straw, or canola straw inhibited seed germination of flixweed, stinkweed, and downy brome, but not wheat. These reports (Moyer & Huang 1997; Huang *et al*. 2002a) suggest that some allelopathic chemicals in certain crop straws may have the potential to control soilborne plant pathogens and weeds. In addition, some agricultural chemicals, such as fertilizers and herbicides, may also have harmful effects on plant pathogens. For example, soil amendments using urea effectively controlled apothecia production from sclerotia of *S. sclerotiorum* (Huang & Janzen 1991). Soil amendment with triazine herbicides, atrazine or simazine, at the recommended rates for weed control resulted in the formation of multiple secondary stipes, which failed to form ascocarps and produce asci and ascospores (Huang & Blackshaw 1995). Therefore, agricultural wastes, agrochemicals, and other industrial wastes may have the potential to develop formulated products for the control of plant diseases.

To develop a formulated compound for disease control, the compound should have the following functions: (1) maximize harmful effects on target plant pathogens, (2) increase the number of beneficial microorganisms, (3) maintain or improve soil fertility, and (4) maintain healthy agroecosystems (Huang & Huang 1993). Most formulated soil amendments are composed of fertilizers, agricultural wastes, and industrial wastes. For example, "S-H Mixture" (R.O.C. Patent No. 021096; Inventors: S. K. Sun and J. W. Huang) is the first commercial product developed in Taiwan to control soilborne diseases in crops, including Fusarium wilt of radish, watermelon and cabbage; club root of Chinese cabbage; bacterial wilt of tomato; Rhizoctonia blight of beans; sheath blight of rice; and southern blight of pepper (Sun & Huang 1985). This product is mainly made from agricultural and industrial wastes such as sugarcane bagasse, rice husk, oyster shell, and mineral ash (Sun & Huang 1985). In addition to controlling soilborne diseases, "S-H Mixture" can also stimulate the growth of beneficial microorganisms in the soil. Another formulated product, "FBN-5A" (R.O.C. Patent No. 135447; Inventors: J. W. Huang and S. S. Yang), mainly consists of

spent forest mushroom compost, fish meal, bone powder, blood powder, rape seed meal, lime, and allyl alcohol (Shiau *et al*. 1999). Adding 0.1% FBN-5A to soil or BVB#4 growth medium can effectively prevent and control damping-off cabbage and radish caused by *Rhizoctonia solani*. In addition to controlling Rhizoctonia damping-off, FBN-5A stimulates the growth of antagonists such as *Trichoderma* spp. (Shiau *et al*. 1999). The third example is the granular biofumigant "PBGG" (R.O.C. Patent No. I276402; Inventors: J. W. Huang, W. C. Chung, H. C. Huang, and J. H. Shiau). It is used to control soilborne pathogens such as *R. solani*, *Pythium* spp., *S. sclerotiorum*, and *Sclerotium rolfsii* (Chung *et al*. 2002; Chung *et al*. 2005). The main ingredients of "PBGG" are *Pseudomonas boreopolis*, Brassica seed pomace, glycerin, and sodium alginate. Application of 1% (w/w) of "PBGG" to soil infested with *R. solani* significantly reduced the percentage of colonization of cabbage seeds by the pathogen and stimulated the growth of actinomycetes, including *Streptomyces padanus* and *S. xantholiticus*. The control of plant diseases is based on the release of fungal toxic substances, isothiocyanates by "PBGG" (Chung *et al*. 2005). These examples illustrate the potential to develop soil-improvement products that inhibit pathogens while promoting the growth and proliferation of biocontrol agents. The disposal of agricultural waste has become an important global environmental issue. Therefore, utilizing agricultural waste to develop effective disease management products will benefit agriculture and society as a whole.

Crop rotation

Crop rotation has long been considered an effective strategy for excluding and eradicating plant diseases (Walker 1969). Repeated monocultures of the same annual crop may lead to disease outbreaks in that crop. For example, results from a long-term (1959–2000) field trial in Hokkaido, Japan, clearly demonstrated the impact of crop rotation on bean diseases and yields (Huang *et al*. 2002c). In this field trial, a total of seven treatments were set up including: (1) bare land (no crops or weeds planted); (2) monoculture (potatoes, sugar beets, oats, kidney beans, or winter wheat); (3) two-year crop rotation (oats, sugar beets); (4) three-year crop rotation (soy bean, sugar beets, oats); (5) four-year crop rotation (potatoes, sugar beets, oats, soybean); (6) five-year crop rotation (sugar beets, oats, winter wheat, red clover, potatoes); and (7) six-year crop rotation (potatoes, sugar beets, oats, kidney beans, winter wheat, red clover). Each treatment was repeated once. Several reports from studies in this field indicate that *Pythium* damping-off (*Pythium* spp.) of beans is severe in monocultures (Kageyama *et al*. 1981; Huang *et al*. 2002c), while bean plants in the six-year rotation treatment are healthy (Huang *et al*. 2002c). Monoculture also causes severe yield losses in bean seeds. From 1989 to 2000, the annual seed yield of beans under monoculture was 970 kg ha^{-1}, while the seed yield in 6-year rotation was 2,380 kg ha^{-1}, resulting in a 59% yield loss in monoculture (Huang *et al*. 2002c). In addition to reduced seed yields, bean monocultures also resulted in reduced seed quality, such as smaller seed size in monoculture plants.

Allelopathy is another important factor to consider when choosing crops for rotation. Moyer & Huang (1997) reported that aqueous extracts of oat, lentil, or canola straws contained allelopathic chemicals that were toxic to flixweed, stinkweed, and downy brome. The healthy bean plants observed in the six-year crop rotation treatment of the long-tern crop rotation trial in Hokkaido, Japan, may be due to the planting sequence of beans after oats (Huang *et al*. 2002c). Other studies in Canada have shown that rotation of legume crops with cereals is superior to cereal monocultures because legume-based rotation systems increase cereal crop yields (Wright 1990), improve soil fertility and quality (Green & Biederbeck 1995), and increase the abundance of soil microorganisms, including fungi, bacteria, and actinomycetes (Biederbeck *et al*. 1999). Therefore, in every

crop rotation plan, it is useful to include legume crops to improve soil fertility and allelopathic crops to control harmful plant pathogens.

Biological control

Many fungal and bacterial plant pathogens have natural enemies or competitors, such as hyperparasites (mycoparasites) or antagonistic microorganisms. These natural enemies of plant pathogens may have potential for biological control of plant diseases. For example, diseases caused by *S. sclerotiorum* are difficult to control because the pathogen has a wide host range, including many important commercial crops, and its black sclerotia survive well even in extreme climatic conditions. Furthermore, black sclerotia of *S. sclerotiorum* can geminate in two different ways: (1) myceliogenic germination to produce mycelia that infect the plant roots, or (2) carpogenic germination to produce air-borne ascospores that infect above-ground tissues of the plant. Therefore, the two sclerotial germination modes of *S. sclerotiorum* may cause distinct crop diseases. For example, sclerotia of *S. sclerotiorum* in the soil germinate myceliogenically to produce mycelia, which infect the roots of sunflowers, causing damping-off of sunflower seedlings (Huang & Dueck 1980) or basal stem rot and wilting of older sunflower plants (Huang & Dueck 1980). On the other hand, when sclerotia in the soil germinate carpogenically to produce apothecia, a large number of ascospores are released into the air and infect the aboveground tissues, causing sunflower head rot and stalk rot, safflower head rot, canola leaf blight, stem blight and pod rot, bean white mold, pea pod rot and alfalfa pod rot (Bardin & Huang 2001).

Studies have shown that *Coniothyrium minitans* is a mycoparasitic fungus, capable of attacking sclerotia (Huang 1977; Huang & Kokko 1987) and hyphae (Huang & Hoes 1976; Huang & Kokko 1988) of *S. sclerotiorum*. *C. minitans* is rich in cell wall degrading enzymes, such as glucanase (Huang *et al*. 2001; Laroche *et al*. 2004), and xylanase (Laroche *et al*. 2000). These *C. minitans* enzymes may play an important role in destroying hyphae and sclerotia of *S. sclerotiorum*. Field trials in western Canada have shown that soil treatment with *C. minitans* can effectively control Sclerotinia wilt of sunflower (Huang 1980), and air spraying of *C. minitans* spores can effectively control bean white mold (Huang *et al*. 2000; McLaren *et al*. 2009) and pea pod rot (Huang & Kokko 1992). In addition to being effective in controlling Sclerotinia diseases, *C. minitans* survives well under the extreme weather conditions of the Canadian prairies (Huang & Erickson 2002). Several studies in western Canada have also shown that compared with other mycoparasites such as *Trichothecium roseum* (Huang & Kokko 1993), *Talaromyces flavus* (McLaren *et al*. 1994, 1996), *Trichoderma virens* (Huang *et al*. 2000) and *Trichoderma atroviride* (Li *et al*. 2005), *C. minitans* is the most effective mycoparasite to control *S. sclerotiorum*. These findings suggest that *C. minitans* is an idea biocontrol agent for controlling Sclerotinia diseases in crops grown in cold regions such as Canada because of the organism's ability to survive cold winters (Huang & Erickson 2002) and can effectively control sclerotia (Huang 1977, 1980) and apothecia (Huang & Erickson 2004a, 2004b) of *S. sclerotiorum* during the crop production season.

The results of research on *C. minitans* as a potential biocontrol agent (Huang 1980; McLaren *et al*. 1994; Huang *et al*. 2000) attracted investment from three Canadian companies- CSP Food Ltd., Saskatoon (1978–1980), PhilomBios, Saskatoon (1986–1988), and Agrium Co., Calgary (1994–1998) to commercialize this mycoparasite; however, all attempts were ultimately unsuccessful. In contrast, commercialization efforts for *C. minitans* have been successful in Europe, including the product Contans WG® from Prophyta, Germany and another product, Koni®, from Bioved Ltd., Hungary (Luth 2001; Whipps & Lumsden 2001). Contans WG® has been distributed to many countries in Europe and North America.

In addition to mycoparasites, many bacterial

species such as *Pseudomonas fluorescens*, *Erwinia* spp., *Streptomyces* spp., and *Rhizobium* spp. are antagonists of many plant pathogens. These antagonistic bacteria may have potential for biocontrol of crop diseases. For example, through indoor screening, *Rhizobium leguminosarum* bv *viceae* "strain R21" was selected and tested for the control of damping-off disease in peas and lentils in fields naturally infested with *Pythium* spp. (Huang & Erickson 2007). The results show that treating pea seeds or lentil seeds with *Rhizobium* "strain R21" can not only control *Pythium* damping-off in peas and lentils, but also increase the seedling height, root nodule size, root biomass, shoot biomass, and seed yield (Huang & Erickson 2007). Although pea seeds treated with the fungicide Thiram™ also effectively controlled *Pythium* damping-off disease, the root nodules produced on pea plants were small in size (Huang & Erickson 2007).

In addition to nitrogen-fixing bacteria, other rhizosphere bacteria have potential as biocontrol agents for crop disease management. For example, seed treatments using *Erwinia rhapontici*, *Pseudomonas fluorescens*, *Bacillus cereus*, or *Pantoea agglomerans* can effectively control *Pythium* damping-off in field crops such as canola, safflower, peas, and sugar beets (Liang *et al*. 1996; Bardin *et al*. 2003). These findings suggest that selected rhizosphere bacterial strains may be developed into biocontrol products. However, before microorganisms are selected for use in the development of biocontrol products, a risk assessment is required to ensure the safety of each biocontrol agent. For example, *E. rhapontici* is an effective agent for controlling *Pythium* damping-off in canola, safflower, peas and sugar beets (Liang *et al*. 1996; Bardin *et al*. 2003), but it is also a plant pathogen, that can cause pink seed disease in many crops, including peas (Huang *et al*. 1990; Huang & Erickson 2004c), beans (Huang *et al*. 2002b; Hsieh *et al*. 2010), lentils (Huang *et al*. 2003a), chickpea (Huang *et al*. 2003a), and wheat (McMullen *et al*. 1984). Therefore, *E. rhapontici* is not suitable for making commercial biocontrol products because it is also a pathogen of many crops (Huang *et al*. 2003b). In comparison, *Rhizobium leguminosarum* bv *viceae* 'strain R21' has great potential for producing commercial products because it not only controls plant diseases but also fixes nitrogen to improve soil fertility (Huang & Erickson 2007).

Culture practice

1. Adjust plant spacing

For soil-borne plant diseases, the location of pathogens in the soil is an important factor in determining disease outbreaks in the field. Taking Sclerotinia wilt of sunflower as an example, Huang & Hoes (1980) conducted field studies and determined the impact of sclerotia of *S. sclerotiorum* buried at different vertical or horizontal distances from sunflower seeds on the incidence of Sclerotinia wilt of sunflower. The results show that the distance between sunflower seeds and sclerotia of *S. sclerotiorum* has a significant impact on the occurrence of Sclerotinia wilt of sunflower. When sclerotia are buried 5 cm deep next to the sunflower seeds, more than 50% of the sunflower plants will be killed and wilted by the pathogen. On the other hand, the incidence of Sclerotinia wilt of sunflower is significantly reduced when the sclerotia are buried 4 cm above the seed or 5 cm or 15 cm below the seed. Likewise, the incidence of Sclerotinia wilt of sunflower is significantly reduced when sunflower seeds and sclerotia are buried at seed level (5 cm deep) but horizontally spaced 10 cm or more apart (Huang & Hoes 1980).

Adjusting plant spacing is an important factor affecting the secondary spread of diseases in the field. Hoes & Huang (1985) conducted a study in a field heavily infested with sclerotia of *S. sclerotiorum*, and they concluded that sunflower wilt due to secondary spread from the primary inoculum (i.e., from sclerotia in the soil) was common at the within-row plant spacing of 15 cm. However, sunflower wilt due to secondary spread from the primary inoculum was rare at the within-row plant spacing of 25 cm. There was no increase in disease due to

plant-to-plant transmission at within-row plant spacing of 36 and 47 cm, and all wilted plants in both treatments were due to primary infection with sclerotia in the soil (Hoes & Huang 1985). Furthermore, studies in this diseased field have shown that sunflower seed yields are maximized with within-row plant spacing of 36 cm or greater, and at 26,000 to 49,000 plants ha^{-1} (Hoes & Huang 1985). This population range also maximizes yields from sunflowers grown on uninfested soils (Robinson *et al*. 1980). Therefore, uniform spacing between sunflower plants can not only maximize sunflower yield (Robinson *et al*. 1982), but also effectively control the secondary spread of Sclerotinia wilt disease in the field (Hoes & Huang 1985).

2. Use of agriculture chemicals

Chemical fertilizers, herbicides, and pesticides are widely used in crop production. Some of these chemicals can harm the environment, including microorganisms. For example, urea is a nitrogen fertilizer for crops, but it is also a fungicide that can control soilborne plant pathogens such as *Fusarium oxysporum* f. sp. *cubense* (Sequeira 1963), *Sclerotium rolfsii* (Fang & Liu 1988) and *Phytophthora* sp. (Tsao & Oster 1981). Huang & Janzen (1991) reported that non-sterile natural soil amended with 100 kg ha^{-1} urea completely inhibited carpogenic germination of sclerotia of *S. sclerotiorum*, but in sterile soil treated with 100 kg ha^{-1} urea, more than 97% of sclerotia germinated carpogenically to produce numerous apothecia. However, if urea-amended, sterile soil is treated with urease, the soil has a strong inhibitory effect on carpogenic germination of sclerotia of *S. sclerotioum*. These findings suggest that ammonia released from the decomposition of urea is a key toxicant that inhibits carpogenic germination of sclerotia of *S. sclerotiorum* (Huang & Janzen 1991). In addition to urea, other chemicals such as calcium cyanamide (Perlka®, https://www.alzchem.com/en/brands/perlka/), not only provide nitrogen and lime to the soil but also control *S. sclerotiorum* (Huang & Sun 1991).

Triazine herbicides, such as atrazine and simazine, are commonly used in crop production in many countries. Canadian studies have shown that triazine herbicides persist in soils in Saskatchewan (Smith & Walker 1989) and Alberta (Moyer & Blackshaw 1993) and that these herbicide residues have reduced yields of subsequent crops in western Canada (Moyer & Blackshaw 1993). Huang & Blackshaw (1995) reported that the triazine herbicides also affect the plant pathogen, *S. sclerotiorum*. Field soils amended with atrazine or simazine at the recommended field application rates (1500 g ha^{-1} or 7.5 ppm) did not stimulate or inhibit carpogenic germination of sclerotia of *S. sclerotiorum*, compared to untreated controls. However, soil treatment with atrazine or simazine results in the formation of secondary stipes on primary stipes that are unable to form normal ascocarps for ascospore production (Huang & Blackshaw 1995). These examples show that certain chemical fertilizers or herbicides may be effective in controlling plant diseases. However, risk assessments are needed to assess the impact of each disease control strategy, including chemical fertilizers, herbicides, and pesticides, on agroecosystems and the environment.

CONCLUSIONS

Agriculture chemicals, including fertilizers, herbicides, and pesticides, are widely used in crop production. There are many examples of heavy reliance on agrochemicals having negatively impacting agricultural ecosystems and the environment. As a result, the “one chemical kills them all” approach to crop production is no longer acceptable in modern agriculture. To ensure the sustainability of crop production, efforts should be made to develop eco-friendly and environmentally sound pest control measures. These include preventive measures, such as phytosanitary measures to prevent the spread of diseases between countries or regions. Crop rotation is another good cultural practice for plant disease management. Nitrogen-fixing legumes and allelopathic crops are good choices for crop rotation. Crop sequence and crop selection are

important factors in determining the success of a crop rotation system. Changing cultural practices, such as irrigation techniques, is an effective way to control plant pathogens that prefer moist, poorly drained soil. Other field management techniques, including the selection of environmentally friendly agricultural chemicals, field sanitation, and insect vector control, can help minimize the impact of crop diseases.

Plant breeding is an effective strategy for plant disease management. Plant breeders should focus on developing new crop varieties with high yield, high quality, and high disease resistance. Biocontrol, using antagonistic or hyperparasitic microorganisms, is considered a good disease management strategy. However, while certain microorganisms may be able to control diseases in certain crops, they may also be harmful plant pathogens in other crops. Therefore, in addition to efficacy trials, risk assessment of the potential harmful effects of biocontrol agents on other crops, farmers, and consumers is crucial to ensure the successful development of biopesticides for practical applications. Commercialization of biocontrol agents remains challenging. More efforts are needed on issues such as formulation, scale-up production, product cost, and user friendliness. Achieving sustainable crop production requires an integrated pest management (IPM) approach that integrates all effective, environmentally friendly disease control measures.

ACKNOWLEDGEMENTS

I would like to thank Dr. Hsueh-Shih Lin, Director of the Taiwan Agriculture Research Institute, Ministry of Agriculture, for inviting me to present the content of this article at the "International Symposium on Biocontrol and Sustainable Cropping Management", June 25, 2024, Taipei, Taiwan. Information on "Commercial biocontrol products developed in Taiwan" was provided by Dr. Jenn-Wen Huang (Emeritus Professor) and Dr. Cheng-Fang Hong (Assistant Professor), National Chung-Hsing University, Taichung, Taiwan.

REFERENCES

Acharya, S. N. and H. C. Huang. 2000. Registration of 'AC Longview' alfalfa. Crop Sci. 40:1823. doi:10.1002/j.1435-0653.2000.tb21498.x

Acharya, S. N. and H. C. Huang. 2003. Breeding alfalfa for resistance to Verticillium wilt: A sound strategy. p.345–371. *in*: Advances in Plant Disease Management. (Huang, H. C. and S. N. Acharya, eds.) Research Signpost, Trivandrum, India. 429 pp.

Acharya, S. N., H. C. Huang, and M. R. Hanna. 1995. Registration of 'AC Blue J' alfalfa. Crop Sci. 35:1225–1226. doi:10.2135/cropsci1995.0011183X003500040084x

Anonymous. 2012. The requirements of import of alfalfa hay from US to China. http://kernag.com/caap/phytos/country/china.pdf (visit on 3/3/2024)

Anonymous. 2021. D-03-14: Canadian hay certification program to certify for export. Effective date: June 4, 2021. 3rd revision. https://inspection.canada.ca/plant-health/invasive-species/directives/grains-and-field-crops/d-03-14/eng/1323829800901/1323829873124#aa3 (visit on 3/3/2024)

Bardin, S. D. and H. C. Huang. 2001. Research on biology and control of Sclerotinia diseases in Canada. Can. J. Plant Pathol. 23:88–98. doi:10.1080/07060660109506914

Bardin, S. D., H. C. Huang, L. Liu, and L. J. Yanke. 2003. Control, by microbial seed treatment, of damping-off caused by *Pythium* sp. on canola, safflower, dry pea, and sugar beet. Can. J. Plant Pathol. 25:268–275. doi:10.1080/07060660309507079

Bergstrom, G. 1984. Verticillium wilt of alfalfa. https://hdl.handle.net/1813/42392 (visit on 10/2/2025)

Biederbeck, V. O., N. Z. Lupwayi, W. A. Rice, K. G. Hanson, and R. P. Zentner. 1999. Crop rotation effects on soil microbial populations, biomass and diversity under wheat in a brown loam. p.594–602. *in*: Proceedings of Soils and Crops. February 25–26, 1999. Saskatoon, Canada. Extension Division, University of Saskatchewan, Saskatoon, Canada.

Boland, G. J. and R. Hall. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 16:93–108. doi:10.1080/07060669409500766

Christen, A. A. 1982. A selective medium for isolating *Verticillium albo-atrum* from soil. Phytopathology 72:47–49. doi:10.1094/Phyto-72-47

Chung, W. C., J. W. Huang, and H. C. Huang. 2005.

Formulation of a soil biofungicide for control of damping-off of Chinese cabbage (*Brassica chinensis*) caused by *Rhizoctonia solani*. Biol. Control 32:287–294. doi:10.1016/j.biocontrol.2004.10.011

Chung, W. C., J. W. Huang, H. C. Huang, and J. F. Jen. 2002. Effect of ground *Brassica* seed meal on control of Rhizoctonia damping-off of cabbage. Can. J. Plant Pathol. 24:211–218. doi:10.1080/07060660309506998

Dorrell, D. G. and H. C. Huang. 1978. Influence of Sclerotinia wilt on seed yield and quality of sunflower wilted at different stages of development. Crop Sci. 18:974–976. doi:10.2135/cropsci1978.0011183X001800060017x

Erickson, R. S. and P. M. Balasubramanian. 2008. Survey of diseases of dry bean in southern Alberta in 2007. Can. Plant Dis. Surv. 88:99–100.

Erickson, R. S. and P. M. Balasubramanian. 2010. Survey of diseases of dry bean in southern Alberta in 2009. Can. Plant Dis. Surv. 90:121–122.

Erickson, R. S. and S. Chatterton. 2012. Survey of diseases of dry bean in southern Alberta in 2011. Can. Plant Dis. Surv. 92:117–118.

Fang, H. C. and T. M. E. Liu. 1988. Effect of urea and nitrogenous compounds on the sclerotial germination and viability of the *Sclerotium rolfsii* in soil and its relationship with soil microorganisms. Plant Prot. Bull. 30:235–244. (in Chinese)

Graham, J. H., R. N. Peaden, and D. W. Evans. 1977. Verticillium wilt of alfalfa found in the United States. Plant Dis. Rep. 61:337–340.

Green, B. J. and V. O. Biederbeck (eds.). 1995. Farm Facts: Soil Improvement with Legumes, Including Legumes in Crop Rotations. Can. Sask. Agrmt. Soil Conserv. Bull. Regina, Canada. 20 pp.

Hanna, M. R. and H. C. Huang. 1987. Barrier alfalfa. Can. J. Plant Sci. 67:827–830. doi:10.4141/cjps87-116

Harper, A. M. and H. C. Huang. 1984. Contamination of insects by the plant pathogen *Verticillium albo-atrum* in an alfalfa field. Environ. Entomol. 13:117–120. doi:10.1093/ee/13.1.117

Hoes, J. A. and H. C. Huang. 1985. Effect of between-row and within-row spacings on development of sclerotinia wilt and yield of sunflower. Can. J. Plant Pathol. 7:98–102. doi:10.1080/07060668509501522

Howard, R. J., H. C. Huang, J. A. Traquair, E. R. Moskaluk, M. J. Kokko, and L. M. Phillippe. 1991. Occurrence of Verticillium wilt of alfalfa in southern Alberta, 1980-86. Can. Plant Dis. Surv. 71:21–27.

Hsieh, T. F., H. C. Huang, and R. S. Erickson. 2010. Spread of seed-borne *Erwinia rhapontici* in bean, pea and wheat. Eur. J. Plant Pathol. 127:579–584. doi:10.1007/s10658-010-9622-0

Hsieh, T. F., H. C. Huang, R. S. Erickson, L. J. Yanke, and H. H. Mündel. 2002. First report of bacterial wilt of common bean caused by *Curtobacterium flaccumfaciens* in western Canada. Plant Dis. 86:1275. doi:10.1094/PDIS.2002.86.11.1275A

Huang, H. C. 1977. Importance of *Coniothyrium minitans* in survival of sclerotia of *Sclerotinia sclerotiorum* in wilted sunflower. Can. J. Bot. 55:289–295. doi:10.1139/b77-039

Huang, H. C. 1980. Control of sclerotinia wilt of sunflower by hyperparasites. Can. J. Plant Pathol. 2:26–32. doi:10.1080/07060668009501458

Huang, H. C. 1989. Distribution of *Verticillium albo-atrum* in symptomed and symptomless leaflets of alfalfa. Can. J. Plant Pathol. 11:235–241. doi:10.1080/07060668909501105

Huang, H. C. 2003. Verticillium wilt of alfalfa: Epidemiology and control strategies. Can. J. Plant Pathol. 25:328–338. doi:10.1080/07060660309507088

Huang, H. C., S. N. Acharya, M. R. Hanna, G. C. Kozub, and E. G. Smith. 1994. Effect of Verticillium wilt on forage yield of alfalfa in Southern Alberta. Plant Dis. 78:1181–1184. doi:10.1094/PD-78-1181

Huang, H. C. and R. E. Blackshaw. 1995. Influence of herbicides on the carpogenic germination of *Sclerotinia sclerotiorum* sclerotia. Bot. Bull. Acad. Sin. 36:59–64.

Huang, H. C., E. Bremer, R. K. Hynes, and R. S. Erickson. 2000. Foliar application of fungal biocontrol agents for the control of white mold of dry bean caused by *Sclerotinia sclerotiorum*. Biol. Control 18:270–276. doi:10.1006/bcon.2000.0829

Huang, H. C., K. J. Cheng, J. L. Zantinge, and A. J. Laroche. 2001. Strains of *Coniothyrium minitans* having β-glucanase activity. U.S. Patent No. 6,268,202 B1. U.S. Patent and Trademark Office. https://patents.google.com/patent/US6268202B1/en (visit on 10/2/2025)

Huang, H. C. and C. H. Chou. 2005. Impact of plant disease biocontrol and allelopathy on biodiversity and agricultural sustainability. Plant Pathol. Bull. 14:1–12. doi:10.6649/PPB.200503_14(1).0001

Huang, H. C. and J. Dueck. 1980. Wilt of sunflower from infection by mycelial-germinating sclerotia of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 2:47–52. doi:10.1080/07060668009501437

Huang, H. C. and R. S. Erickson. 1994a. Survey of diseases of dry beans in southern Alberta in 1993. Can. Plant Dis. Surv. 74:98–99.

Huang, H. C. and R. S. Erickson. 1994b. Survey of

Verticillium wilt of alfalfa in southern Alberta in 1993. Can. Plant Dis. Surv. 74:59.

Huang, H. C. and R. S. Erickson. 1995. Survey of diseases of alfalfa in southern Alberta in 1994. Can. Plant Dis. Surv. 75:162–163.

Huang, H. C. and R. S. Erickson. 1999. Survey of diseases of dry bean in southern Alberta in 1998. Can. Plant Dis. Surv. 79:111–112.

Huang, H. C. and R. S. Erickson. 2000. Survey of diseases of dry bean in southern Alberta in 1999. Can. Plant Dis. Surv. 80:73–74.

Huang, H. C. and R. S. Erickson. 2002. Overwintering of *Coniothyrium minkans*, a mycoparasite of *Sclerotinia sclerotiorum*, on the Canadian prairies. Australasian Plant Pathol. 31:291–293. doi:10.1071/AP02019

Huang, H. C. and R. S. Erickson. 2004a. Control of white mold of bean by *Coniothyrium minitans*: Comparison of soil and foliar treatments. Plant Pathol. Bull. 13:171–176. doi:10.6649/PPB.200409_13(3).0001

Huang, H. C. and R. S. Erickson. 2004b. Effect of soil treatment of fungal agents on control of apothecia of *Sclerotinia sclerotiorum* in canola and safflower fields. Plant Pathol. Bull. 13:1–6. doi:10.6649/PPB.200403_13(1).0001

Huang, H. C. and R. S. Erickson. 2004c. Impact of pink seed of pea caused by *Erwinia rhapontici* in Canada. Plant Pathol. Bull. 13:261–266. doi:10.6649/PPB.200412_13(4).0003

Huang, H. C. and R. S. Erickson. 2007. Effect of seed treatment with *Rhizobium leguminosarum* on *Pythium* damping-off, seedling height, root nodulation, root biomass, shoot biomass, and seed yield of pea and lentil. J. Phytopathology 155:31–37. doi:10.1111/j.1439-0434.2006.01189.x

Huang, H. C., R. S. Erickson, C. Chang, J. R. Moyer, F. J. Larney, and J. W. Huang. 2002a. Organic soil amendments for control of apothecial production of *Sclerotinia sclerotiorum*. Plant Pathol. Bull. 11:207–214. doi:10.6649/PPB.200212_11(4).0006

Huang, H. C., R. S. Erickson, T. F. Hsieh, R. L. Conner, and P. M. Balasubramanian. 2009. Resurgence of bacterial wilt of common bean in North America. Can. J. Plant Pathol. 31:290–300. doi:10.1080/07060660909507603

Huang, H. C., R. S. Erickson, L. J. Yanke, T. F. Hsieh, and R. A. A. Morrall. 2003a. First report of pink seed of lentil and chickpea caused by *Erwinia rhapontici* in Canada. Plant Dis. 87:1398. doi:10.1094/PDIS.2003.87.11.1398A

Huang, H. C., R. S. Erickson, L. J. Yanke, H. H. Mundel, and T. F. Hsieh. 2002b. First report of pink seed of common bean caused by *Erwinia rhapontici*. Plant Dis. 86:921. doi:10.1094/PDIS.2002.86.8.921C

Huang, H. C. and M. R. Hanna. 1991. An efficient method to evaluate alfalfa cultivars for resistance to Verticillium wilt. Can. J. Plant Sci. 71:871–875. doi:10.4141/cjps91-126

Huang, H. C., M. R. Hanna, and E. G. Kokko. 1985. Mechanisms of seed contamination by *Verticillium albo-atrum* in alfalfa. Phytopathology 75:482–488. doi:10.1094/Phyto-75-482

Huang, H. C. and A. M. Harper. 1985. Survival of *Verticillium albo-atrum* from alfalfa in feces of leaf-chewing insects. Phytopathology 75:206–208. doi:10.1094/Phyto-75-206

Huang, H. C., A. M. Harper, E. G. Kokko, and R. J. Howard. 1983. Aphid transmission of *Verticillium albo-atrum* to alfalfa. Can. J. Plant Pathol. 5:141–147. doi:10.1080/07060668309501615

Huang, H. C., R. Hironaka, and R. J. Howard. 1986a. Survival of *Verticillium albo-atrum* in alfalfa tissue buried in manure or fed to sheep. Plant Dis. 70:218–221. doi:10.1094/PD-70-218

Huang, H. C. and J. A. Hoes. 1976. Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minitans*. Can. J. Bot. 54:406–410. doi:10.1139/b76-039

Huang, H. C. and J. A. Hoes. 1980. Importance of plant spacing and sclerotial position to development of Sclerotinia wilt of sunflower. Plant Dis. 64:81–84. doi:10.1094/PD-64-81

Huang, H. C., T. F. Hsieh, and R. S. Erickson. 2003b. Biology and epidemiology of *Erwinia rhapontici*, causal agent of pink seed and crown rot of plants. Plant Pathol. Bull. 12:69–76. doi:10.6649/PPB.200306_12(2).0001

Huang, H. C. and J. W. Huang. 1993. Prospects for control of soilborne plant pathogens by soil amendment. Curr. Top. Bot. Res. 1:223–235.

Huang, H. C. and H. H. Janzen. 1991. Control of carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* by volatile substances from urea. Plant Prot. Bull. 33:283–289.

Huang, H. C., F. Kodama, K. Akashi, and K. Konno. 2002c. Impact of crop rotation on soilborne diseases and yield of kidney bean: A case study in northern Japan. Plant Pathol. Bull. 11:87–96. doi:10.6649/PPB.200206_11(2).0005

Huang, H. C. and E. G. Kokko. 1985. Infection of alfalfa pollen by *Verticillium albo-atrum*. Phytopathology 75:859–865.

Huang, H. C. and E. G. Kokko. 1987. Ultrastructure of hyperparasitism of *Coniothyrium minitans* on sclerotia of *Sclerotinia sclerotiorum*. Can. J. Bot. 65:2483–2489. doi:10.1139/b87-337

Huang, H. C. and E. G. Kokko. 1988. Penetration of hyphae of *Sclerotinia sclerotiorum* by *Coniothyrium minitans*

without the formation of appressoria. J. Phytopathology 123:133–139. doi:10.1111/j.1439-0434.1988.tb04460.x

Huang, H. C. and E. G. Kokko. 1992. Pod rot of dry peas due to infection by ascospores of *Sclerotinia sclerotiorum*. Plant Dis. 76:597–600. doi:10.1094/PD-76-0597

Huang, H. C. and E. G. Kokko. 1993. *Trichothecium roseum*, a mycoparasite of *Sclerotinia sclerotiorum*. Can. J. Bot. 71:1631–1638. doi:10.1139/b93-198

Huang, H. C., M. J. Kokko, and L. M. Phillippe. 1988. White mold of dry bean (*Phaseolus vulgaris* L.) in southern Alberta, 1983–87. Can. Plant Dis. Surv. 68:11–13.

Huang, H. C., H. H. Marshall, L. M. Collicutt, D. L. McLaren, and M. J. Kokko. 1993. Screening hardy chrysanthemums for resistance to *Fusarium oxysporum* f. sp. *chrysanthemi* Race 2. Plant Pathol. Bull. 2:103–105. doi:10.6649/PPB.199306_2(2).0007

Huang, H. C., R. J. Morrison, H. H. Muendel, D. J. S. Barr, G. R. Klassen, and J. Buchko. 1992a. *Pythium* sp. "group G", a form of *Pythium ultimum* causing damping-off of safflower. Can. J. Plant Pathol. 14:229–232. doi:10.1080/07060669209500881

Huang, H. C. and L. M. Phillippe. 1989. Survey of dry bean for Sclerotinia white mold in southern Alberta in 1988. Can. Plant Dis. Surv. 69:53.

Huang, H. C., L. M. Phillippe, H. H. Marshall, L. M. Collicutt, and G. A. Neish. 1992b. Wilt of hardy chrysanthemum caused by a new race of *Fusarium oxysporum* f. sp. *chrysanthemi*. Plant Pathol. Bull. 1:57–61. doi:10.6649/PPB.199206_1(2).0002

Huang, H. C., L. M. Phillippe, and R. C. Phillippe. 1990. Pink seed of pea: A new disease caused by *Erwinia rhapontici*. Can. J. Plant Pathol. 12:445–448. doi:10.1080/07060669009500990

Huang, H. C. and K. W. Richards. 1983. *Verticillium albo-atrum* contamination on leaf pieces forming cells for the alfalfa leafcutter bee. Can. J. Plant Pathol. 5:248–250. doi:10.1080/07060668309501605

Huang, H. C., K. W. Richards, and E. G. Kokko. 1986b. Role of the leafcutter bee in dissemination of *Verticillium albo-atrum* in alfalfa. Phytopathology 76:75–79. doi:10.1094/Phyto-76-75

Huang, H. C., G. Saindon, and R. S. Erickson. 1995. Survey of diseases of dry beans in southern Alberta in 1994. Can. Plant Dis. Surv. 75:131–132.

Huang, H. C., G. Saindon, R. S. Erickson, and P. Ma. 1996. Survey of diseases of dry beans in southern Alberta in 1995. Can. Plant Dis. Surv. 76:93–94.

Huang, H. C. and S. K. Sun. 1991. Effect of S-H mixture or Perlka™ on carpogenic germination and survival of sclerotia of *Sclerotinia sclerotiorum*. Soil Biol. Biochem. 23:809–813. doi:10.1016/0038-0717(91)90090-7

Kageyama, K., T. Ui, and Y. Narita. 1981. Influence of *Pythium* spp. on the injury by bean monoculture. Ann. Phytopathol. Soc. Jpn. 47:320–326. (in Japanese with English abstract) doi:10.3186/jjphytopath.47.320

Laroche, A. J., T. Y. Huang, M. M. Frick, Z. X. Lu, H. C. Huang, and K. J. Cheng. 2000. *Coniothyrium minitans* xylanase gene cxy1. U.S. Patent No. 6,121,034 A. U.S. Patent and Trademark Office. https://patents.google.com/patent/US6121034A/en (visit on 10/2/2025)

Laroche, A. J., T. Y. Huang, M. M. Frick, Z. X. Lu, H. C. Huang, and K. J. Cheng. 2004. *Coniothyrium minitans* β-(1, 3) exoglucanase gene cbeg1. U.S. Patent No. 6,734,344 B2. U.S. Patent and Trademark Office. https://patents.google.com/patent/US6734344B2/en (visit on 10/2/2025)

Li, G. Q., H. C. Huang, S. N. Acharya, and R. S. Erickson. 2005. Effectiveness of *Coniothyrium minitans* and *Trichoderma atroviride* in suppression of sclerotinia blossom blight of alfalfa. Plant Pathol. 54:204–211. doi:10.1111/j.1365-3059.2005.01119.x

Liang, X. Y., H. C. Huang, L. J. Yanke, and G. C. Kozub. 1996. Control of damping-off of safflower by bacterial seed treatment. Can. J. Plant Pathol. 18:43–49. doi:10.1080/07060669609500653

Luth, P. 2001. The biological fungicide Contans WG®- A preparation on the basis of the fungus *Coniothyrium minitans*. p.127–128. *in*: Proceedings of Sclerotinia 2001. The XI International Sclerotinia Workshop. July 8–12, 2001. York, UK. British Society for Plant Pathology. London, UK.

McDonald, J. G. and E. Wong. 2000. High diversity in *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* characterized by serology and rep-PCR genomic fingerprinting. Can. J. Plant Pathol. 22:17–22.

McLaren, D. L., H. C. Huang, R. L. Conner, T. L. Henderson, R. S. Erickson, and X. He. 2009. Foliar application strategies and use of pollen with *Coniothyrium minitans* to control white mold of bean, caused by *Sclerotinia sclerotiorum*, in western Canada. Plant Prot. Bull. 51:1–18.

McLaren, D. L., H. C. Huang, G. C. Kozub, and S. R. Rimmer. 1994. Biological control of Sclerotinia wilt of sunflower with *Talaromyces flavus* and *Coniothyrium minitans*. Plant Dis. 78:231–235. doi:10.1094/PD-78-0231

McLaren, D. L., H. C. Huang, and S. R. Rimmer. 1996. Control of apothecial production of *Sclerotinia sclerotiorum* by *Coniothyrium minitans* and *Talaromyces flavus*. Plant Dis. 80:1373–1378. doi:10.1094/PD-80-1373

McMullen, M. P., R. W. Stack, J. D. Miller, M. C. Bromel, and V. L. Youngs. 1984. *Erwinia rhapontici*, a bacterium causing pink wheat kernels. Proc. North Dakota Acad. Sci. 38:78.

Moyer, J. R. and R. E. Blackshaw. 1993. Effect of soil moisture on atrazine and cyanazine persistence and injury to subsequent cereal crops in southern Alberta. Weed Technol. 7:988–994. doi:10.1017/S0890037X00038124

Moyer, J. R. and H. C. Huang. 1997. Effect of aqueous extracts of crop residues on germination and seedling growth of ten weed species. Bot. Bull. Acad. Sin. 38:131–139.

Organization Europeennee et Mediterraneenne pour la Protection des Plants-European Plant Protection Organization (OEPP/EPPO). 1982. Data sheets on quarantine organisms No 48. *Corynebacterium flaccumfaciens* pv. *flaccumfaciens*. Bull. OEPP 12(1).

Patrick, Z. A. 1986. Allelopathic mechanisms and their exploitation for biological control. Can. J. Plant Pathol. 8:225–228. doi:10.1080/07060668609501831

Robinson, R. G., J. H. Ford, W. E. Lueschen, D. L. Rabas, L. J. Smith, D. D. Warnes, and J. V. Wiersma. 1980. Response of sunflower to plant population. Agron. J. 72:869–871. doi:10.2134/agronj1980.00021962007200060003x

Robinson, R. G., J. H. Ford, W. E. Lueschen, D. L. Rabas, D. D. Warnes, and J. V. Wiersma. 1982. Response of sunflower to uniformity of plant spacing. Agron. J. 74:363–365. doi:10.2134/agronj1982.00021962007400020024x

Sato, R. 1994. Outbreak of alfalfa Verticillium wilt in Hokkaido. Japan. Agric. Res. Quarterly. 28:44–51.

Sequeira, L. 1963. Effect of urea applications on survival of *Fusarium oxysporum* f. *cubense* in soil. Phytopathology 53:332–336.

Sheppard, J. W. 1979. Verticillium wilt, a potentially dangerous disease of alfalfa in Canada. Can. Plant Dis. Surv. 59:60.

Sheppard, J. W. and S. N. Needham. 1980. Verticillium wilt of alfalfa in Canada: Occurrence of seed-borne inoculum. Can. J. Plant Pathol. 2:159–162.

Shiau, F. L., W. C. Chung, J. W. Huang, and H. C. Huang. 1999. Organic amendment of commercial culture media for improving control of Rhizoctonia damping-off of cabbage. Can. J. Plant Pathol. 21:368–374.

Smith, A. E. and A. Walker. 1989. Prediction of the persistence of the triazine herbicides atrazine, cyanazine, and metribuzin in Regina heavy clay. Can. J. Soil Sci. 69:587–595. doi:10.4141/cjss89-059

Smith, E. G., S. N. Acharya, and H. C. Huang. 1995. Economics of growing Verticillium wilt-resistant and adapted alfalfa cultivars in western Canada. Agron. J. 87:1206–1210. doi:10.2134/agronj1995.00021962008700060028x

Sun, S. K. and J. W. Huang. 1985. Formulated soil amendment for controlling Fusarium wilt and other soilborne diseases. Plant Dis. 69:917–920.

Tegli, S., A. Sereni, and G. Surico. 2002. PCR-based assay for the detection of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in bean seeds. Lett. Appl. Microbiol. 35:331–337. doi:10.1046/j.1472-765x.2002.01187.x

Tsao, P. H. and J. J. Oster. 1981. Relation of ammonia and nitrous acid to suppression of *Phytophthora* in soils amended with nitrogenous organic substances. Phytopathology 71:53–59. doi:10.1094/Phyto-71-53

Walker, J. C. 1969. Plant Pathology. 3rd ed. McGraw-Hill Book. New York, NY. 819 pp.

Whipps, J. M. and R. D. Lumsden. 2001. Commercial use of fungi as plant disease biological control agents: Status and prospects. *in*: Fungi as Biocontrol Agents: Progress, Problems and Potential. (Butt, T. M., C. Jackson, and N. Magan, eds.) CABI. Wallingford, UK. 390 pp. doi:10.1079/9780851993560.0009

Wright, A. T. 1990. Yield effect of pulses on subsequent cereal crops in the northern prairies. Can. J. Plant Sci. 70:1023–1032. doi:10.4141/cjps90-125

永續農業之植物病害管理策略

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摘要

黃鴻章。2026。永續農業之植物病害管理策略。台灣農業研究 75(2):185–199。

作物病害的發生與蔓延，乃是植物病原、寄主植物及環境條件三者交互作用的結果。不當的栽培管理措施，例如使用帶病菌種子、選用感病品種或採取不合宜的耕作方式，皆可能導致作物病害的發生或再度爆發。病害管理在現代農業中具有極其重要的地位，在當前強調節能與環保的時代，所有作物病害防治方法皆須兼具高效、經濟與環境友善等特性。病害管理措施，如使用無病原種子、抗病品種、輪作制度、生物防治、土壤改善及良好耕作管理等，皆被視為合成化學農藥的可行替代方案。本文進一步以真菌與細菌性病害為例，探討這些環境友善型植物病害防治方法的發展前景與應用。

關鍵詞：相剋作用、生物防治、輪作、抗病性、生態學、病害流行學、土壤改善、昆蟲與病原關係。

投稿日期：2025 年 10 月 2 日；接受日期：2026 年 1 月 28 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):201–216 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0003

Research Article

Taxonomic Revision of the Genus *Sinocrepis* Chen, 1933 (Coleoptera: Chrysomelidae: Galerucinae: Alticitae)

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Abstract

Lee, C. F. and M. Y. Chiang. 2026. Taxonomic revision of the genus *Sinocrepis* Chen, 1933 (Coleoptera: Chrysomelidae: Galerucinae: Alticitae). J. Taiwan Agric. Res. 75(2):201–216.

The species-group names of the genus *Sinocrepis* Chen, 1933, were re-evaluated. *Sinocrepis micans* Chen, 1933, as a junior synonym of *S. obscurofasciata* is supported by examining types and genital characters from a large number of specimens. In addition, *S. nigripennis*, Chen 1936, and *S. fulva* Kimoto, 1996 are herein synonymized with *S. obscurofasciata*. A new species, *S. kimotoi*, sp. nov. is described based on two specimens from Thailand that were misidentified as *S. obscurofasciata*. New data on the biology of *S. obscurofasciata* are provided.

Key words: Leaf beetle, Malvaceae, *Hibiscus tiliaceus*, *Hibiscus taiwanensis*, *Malvaviscus arboreus*.

INTRODUCTION

The monotypic genus *Sinocrepis* was established by Chen (1933) based on *S. micans* Chen, 1933 from China. This type species was synonymized with *Crepidodera obscurofasciata* Jacoby, 1892 from Myanmar by Scherer (1969), who also transferred the latter species to *Sinocrepis*, thereby maintaining the validity of the genus. Subsequently, *S. nigripennis* Chen, 1936, the second species, was described from Sri Lanka. Kimoto (1996) described the third and final species, *S. fulva* Kimoto, 1996 from Taiwan.

Adults of the genus *Sinocrepis* can be recognized by a combination of the following characters: pronotum and elytra glabrous; pronotum with ante-basal impression, with ends bounded by a short, longitudinal impression extending to basal margin, length of ante-basal impression more than 1/2 width of pronotum (Figs. 1A, 1E, 1F, 1H); postantennal tubercles short, not extending to interantennal space (Fig. 1I); elytron with indistinct, closely placed rows of punctures; hind tibia with a simple apical spine (Yang *et al*. 2015).

Sinocrepis obscurofasciata is a common and widespread species in East and South Asia. It was recorded from Taiwan by Chen (1934) for the first time (as *S. micans*), followed by many subsequent records (Chûjô 1935, 1963a, 1963b; Kimoto 1966, 1970, 1986, 1987, 1989, 1991). The species also occurs in Myanmar, India, and Sri Lanka (Scherer 1969), Laos (Medvedev 2009), Vietnam (Medvedev 2015), and Thailand (Kimoto 2000).

Kimoto (1996) described *Sinocrepis fulva* as new, mainly based on its distinctive yellow coloration from the bicolored form of *S. obscurofasciata* in Taiwan, without comparing their aedeagi. The yellow color pattern also appears on types of *S. obscurofasciata*. In addition, *S. nigripennis* is likely a synonym of *S. obscurofasciata*, since both species are sympatric

Received: February 23, 2026; Accepted: March 12, 2026.
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in Sri Lanka. In the present study, we determined the validity of these species by examining their type specimens, voucher specimens, and other available materials, with emphasis on the aedeagal features, and provided new biological information for *S. obscurofasciata*.

MATERIALS AND METHODS

In total, 524 specimens were available for the present study, including 131 specimens obtained through extensive collections made by the Taiwan Chrysomelid Research Team (TCRT) (Lee 2025), and 294 specimens were collected and deposited at the Taiwan Agricultural Research Institute during 1979–1984. At least three males and females from each species were examined to delimit variability of diagnostic characters. For species collected from more than one locality or exhibiting color variations, at least one pair of each sex from each locality and color morph was examined. Length was measured from the anterior margin of the eye to the elytral apex, and width at the greatest width of the elytra. Terminology for adult morphological structures follows Duckett & Daza (2004). Names of plant species follow "Taiwan Encyclopedia of Life, TaiEOL" (TaiEOL n.d.). Specimens studied herein are deposited at the following institutes and collections:

(1) KMNH: Kitakyushu Museum of Natural History and Human History, Kitakyushu, Japan [Yûsuke Minoshima];
(2) MCSN: Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy [Roberto Poggi];
(3) MNHN: Muséum national d'Histoire naturelle, Paris, France [Christophe Rivier, Antoine Mantilleri];
(4) NHMUK: The Natural History Museum, London, UK [Michael F. Geiser];
(5) NMNS: National Museum of Natural Science, Taichung, Taiwan [Bao-Cheng Lai];
(6) SDEI: Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany [Mandy Schröter];
(7) SEHU: Laboratory for Systematic Entomology, Hokkaido University, Sapporo, Japan [Munetoshi Maruyama];
(8) TARI: Applied Zoology Division, Taiwan Agricultural Research Institute, Taichung, Taiwan [Chi-Feng Lee].

Label data of type specimens are cited verbatim, with a double slash (//) dividing the data on different labels and a single slash (/) denoting those on different lines on the same label. Other comments and remarks are in square brackets: [p]- preceding data are printed, [h]- preceding data are handwritten, [w]- white label, [b]- blue label, [g]- green label, and [r]- red label. Traditional Chinese fonts are added to the names of localities.

RESULTS

Sinocrepis obscurofasciata (Jacoby)

Crepidodera obscurofasciata Jacoby, 1892: 933 (Myanmar).

S. obscurofasciata: Scherer 1969: 119 (India, Sri Lanka); Kimoto 1986: 60 (Taiwan); Kimoto 1987: 191 (Taiwan); Kimoto 1989: 262 (Taiwan); Kimoto 1991: 18 (Taiwan including Ludao Island 綠島); Medvedev 2009: 167 (Laos); Medvedev 2015: 72 (Vietnam); Kimoto 2000: 272 (Thailand, in part); Ruan & Yang 2017: 220 (China: Shaanxi).

S. micans Chen, 1933: 233 (China: Guizhou); Chen 1934: 181 (Taiwan); Chûjô 1935: 462 (Taiwan including Lanyu Island 蘭嶼); Chûjô 1963a: 402 (Taiwan); Chûjô 1963b: 28 (Taiwan); Gressitt & Kimoto 1963: 760 (China: Hubei, Jiangxi, Zhejiang); Kimoto 1966: 35 (Taiwan); Kimoto 1970: 216 (Taiwan). Synonymized with *S. obscurofasciata* by Scherer (1969).

Sinocrepis nigripennis Chen, 1936: 85 (Sri Lanka). **syn. nov.**

Sinocrepis fulva Kimoto, 1996: 46 (Taiwan). **syn. nov.**

Types. *Crepidodera obscurofasciata*. Lectotype ♂ (Figs. 1A–C) (MCSN, here designated for clarifying its taxonomic status): "Bhamò / Birmania / Fea [p] VII [h] 188[p]5 [h,

w] // Crepidodera / obscurofasciata / Jac. [h, b] // obscurofasciata / Jac. [h, w] // SYNTYPUS [p] / Crepidodera / obscurofasciata / Jacoby, 1892 [h, r] // Museo Civico / di Genova [p, w]”. Paratype. 1♀ (MCSN): “Bhamò / Birmania / Fea [p] VII [h] 188[p]5 [h, w] // SYNTYPUS [p] / Crepidodera / obscurofasciata / Jacoby, 1892 [h, r] // Museo Civico / di Genova [p, w]”.

Sinocrepis nigripennis. Lectotype ♂ (SDEI, here designated for clarifying its taxonomic status): “Anuradhapura / W. Horn 1899 [p, w] // Ohen det. [p, w] // Sinocrepis / nigripennis [h] / S. H. CHEN det. [p, w] // Syntypus [p, r] // SDEI Münchebrg / Col – 24240 [p, g]”. Paralectotypes. 1♂, 3♀ (SDEI) (Fig. 1E), same data as lectotype, but lacking determination label, with “24235, 24239 (Fig. 1D), 24241, or 24242”; 1♀ (SDEI): “Nalanda / W. Horn 1899 [p, w] // Sinocrepis / nigripennis / n. sp. [h, w] // Syntypus [p, r] // SDEI Münchebrg / Col – 24236 [p, g]”; 2♀ (SDEI) “Nalanda / W. Horn 1899 [p, w] // Syntypus [p, r] // SDEI Münchebrg / Col – 24237 or 24238 [p, g]”.

Sinocrepis micans. Lectotype ♂ (Figs. 1H–K) (MNHN, here designated for clarifying its taxonomic status): “MUSEUM PARIS / KOUY-TCHÉOU / RÉG. DE PIN-FA / PÈRE CAVALERIE 1909 [p, w] // Sinocrepis / micans / n. g. n. sp. [h] / S. H. CHEN det. [p, w] // TYPE [p, w, red letters] // SYNTYPE [p, r] // SYNTYPE / Sinocrepis / micans Chen, 1933 [p, w] // MNHN, Paris / EC56910 [p, w]”. There should be another syntype deposited at the MNHN according to the original description (Chen 1933).

Sinocrepis fulva. Paratype. 1♀ (KMNH): “(Taiwan) / Oluanpi (= 鵝鑾鼻) / Pingtung [p, w] // 20[h]. iii.1966 / K. Yano et / H. Kajita [p, w] // Japan-U. S. / Co-op. Sci. / Programme [p, y] // PARATOPOTYPE [p, b] // Sinocrepis / fulva / Kimoto, n. sp. [h] / Det. S. Kimoto 19 [p, w]”. This is the only type found at the KMNH. According to the original description (Kimoto 1996), the holotype and one additional paratype were not found at the SEHU, where both types were deposited.

Specimens examined. CHINA. Guandong: 1♂, 1♀ (TARI), Shenzhen (深圳), 12.III.2019, leg. M.-B. Jiang; Hubei: 1♂ (KMNH), Sulho, Lichuan (利川), 14.IX.1948, leg. Gressitt & Djou; Zhejiang: 2♂ (NHMUK), Haining (海寧), leg. J. J. W., G. C. Champion Coll., B.M.1927-409; **LAOS.** Phongsaly: 1♂ (NHMUK), Phongsaly env., Phu Fa, 1,450–1,600 m, 26.VII.2006, leg. M. Geiser; **TAIWAN.** Chiayi: 1♂, 2♀ (TARI), Arisan (= Alishan, 阿里山), 2-23.X.1918, leg. J. Sonan; 1♂ (NMNS), Alishan Tapan (達邦), 14.II.1989, leg. K. W. Huang; Hsinchu: 10♂, 12♀ (TARI), Wufeng (五峰), 400 m, 14-16.VII.1982, leg. K. C. Chou & C. C. Pan; Hualien: 1♂ (TARI), Antung (安通), 14-17.II.1984, leg. K. C. Chou & C. C. Pan; 1♂ (TARI), Karenko (= Hualien, 花蓮), 20.VII.-4.VIII.1919, leg. T. Okuni; Kaohsiung: 1♂, 3♀ (TARI), Liukuei (六龜), 27-28.VI.1981, leg. L. Y. Chou & C. H. Yang; 1♂, 1♀ (TARI), Tengchih (藤枝), 2-5.VI.2008, leg. C.-F. Lee; Kinmen: Kinmen Island, 1♀ (TARI), Chungshanlin (中山林), 28.VI.2023, leg. C.-F. Lee; same island, 3♂, 6♀ (TARI), Tzuhu (慈湖), 14.VI.2018, H.-D. Fang; Lieyu Island, 10♂, 6♀ (TARI), Hsishanhu (習山湖), 13.X.2022, leg. T.-C. Wang; 5♂, 9♀ (TARI), same locality, 16.II.2023, leg. C.-F. Lee; 3♂, 4♀ (TARI), same but with “12,VII.2023”; Miaoli: 1♂, 2♀ (NHMUK), Daan River SW of Sanyi (三義), 150 m, 26-28.X.2008, leg. L. Dembický; Nantou: 1♂ (TARI), Hoshe (和社), 1,000 m, 22.VII.1982, leg. L. Y. Chou & T. Lin; 28♂, 22♀ (TARI), Lushan (廬山), 1,000 m, 27-31.V.1980, leg. K. S. Lin & L. Y. Chou; 1♂ (TARI), Meifeng (梅峰), 2,150 m, 8.VI.1980, leg. K. S. Lin & B. H. Chen; 1♂ (TARI), same locality, 24-26.VI.1981, leg. K. S. Lin & W. S. Tang; 1♂ (TARI), same locality, 4-7.X.1982, leg. K. C. Chou; 1♀ (TARI), Musha (= Wushe, 霧社), 1,150 m, 18.V.-15.VI.1919, leg. T. Okuni; 1♂, 1♀ (TARI), same locality, 23-28.VI.1981, leg. K. S. Lin & W. S. Tang; 1♂ (TARI), same locality, 6.XI.1981, leg. S. C. Lin & W. S. Tang; 1♂ (TARI), same locality, 14.VII.1982, leg. S. C. Lin & C. N. Lin; 7♂, 2♀ (TARI), same locality, 30.VIII.-2.IX.1982, leg. L. Y. Chou & K. C. Chou; 1♂, 1♀ (TARI), same locality,

7-8.X.1982, leg. K. C. Chou; 2♂, 2♀ (TARI), same but with "17.VIII.1984"; 1♂ (KMNH), Puli (埔里), 11.VII.1966, leg. H. Kamiya; 2♂, 3♀ (TARI), Tsuifeng (翠峰), 2,300 m, 25-27.VI.1981, leg. K. S. Lin & W. S. Tang; 7♂, 5♀ (TARI), Tungpu (東埔), 1,200 m, 25-29.IX.1980, leg. L. Y. Chou & T. Lin; 7♂, 3♀ (TARI), same locality, 28.IV.-2.V.1981, leg. T. Lin & C. J. Lee; 8♂, 3♀ (TARI), same locality, 5-8.X.1981, leg. T. Lin & W. S. Tang; 12♂, 9♀ (TARI), same locality, 18-23.XI.1981, leg. T. Lin & W. S. Tang; 1♂, 2♀ (TARI), same locality, 19-23.VII.1982, leg. L. Y. Chou & T. Lin; 2♀ (TARI), same locality, 22-25.XI.1982, leg. K. C. Chou & S. P. Huang; 8♂, 8♀ (TARI), same locality, 20-24.VI.1983, leg. K. C. Chou & C. Y. Wong; 2♂, 2♀ (TARI), same locality, 23-27.VII.1984, leg. K. C. Chou & C. H. Yang; New Taipei: 2♀ (TARI), Linshanpi (麟山鼻), 15.IX.2007, leg. M.-H. Tsou; 1♀ (TARI), Yehliu (野柳), 6.XI.2008, leg. S.-F. Yu; Pingtung: 7♀ (TARI), Chuanfanshih (船帆石), 16.I.2010, leg. C.-F. Lee; 1♂, 2♀ (TARI), Koshun (= Hengchun, 恆春), 25.IV.-25.V.1918, leg. J. Sonan; 1♂, 2♀ (TARI), Kuaru (= Sheding, 社頂), 15.VI.1937, leg. M. Chujo; 1♂ (TARI), same locality, 2-6.VIII.1982, leg. K. C. Chou & C. N. Lin; 3♂ (TARI), Kuraru (= Sheding, 社頂), 21-25.VIII.1932, leg. Y. Miwa; 4♂, 4♀ (TARI), same locality, 1-4.III.1956, leg. K. S. Lin; 1♀ (TARI), same but with "15.V.1933"; 6♂, 3♀ (TARI), Lungkeng (龍坑), 29.III.2010, leg. J.-C. Chen; 2♂, 2♀ (TARI), Shantimen (三地門), 1-5.III.1982, leg. K. C. Chou & C. C. Pan; 3♂, 1♀ (TARI), Tsaopu (草埔), 24.II.2024, leg. J.-C. Chen; 2♂, 2♀ (TARI), Wanliteshan (萬里得山), 20.II.2007, leg. S.-F. Yu; Taichung: 4♂, 6♀ (TARI), Chiapaotai (加保台), 750 m, 14-18.X.1980, leg. K. S. Lin & C. H. Wang; 41♂, 55♀ (TARI), Kukuan (谷關), 730 m, 20-22.VI.1978, leg. K. S. Lin & K. C. Chou; 11♂, 6♀ (TARI), same locality, 14-17.X.1980, leg. K. S. Lin & C. H. Wang; 3♂, 6♀ (TARI), Wanfeng Hill (萬豐), 4-8.VII.1981, leg. W. S. Tang; Tainan: 4♂ (NMNS), Chingshan (青山), 18.IV.1992, leg. C. Y. Li; Taipei: 8♂, 15♀ (TARI), Chihshanyan (芝山岩), 11.VIII.2007, leg. M.-H. Tsou; 1♂ (TARI), Chutzuhu (竹子湖), 25.IX.2007, leg. S.-F. Yu; 1♀ (NMNS), Inner Lake (內湖), 12-14.IX.1988, leg. K. S. Huang; 1♀ (TARI), Kueitzukeng (貴子坑), 3.VIII.2006, leg. H.-T. Cheng; 2♂, 4♀ (TARI), Peitou (北投), 31.XII.2006, leg. M.-H. Tsou; 1♂, 1♀ (TARI), Taihoku (= Taipei City, 臺北市), 4.XI.1915, leg. M. Maki; 2♂, 2♀ (TARI), same locality, IX.1911, leg. I. Nitobe; 1♂, 1♀ (TARI), same locality, 11.VII.1932, leg. S. Minowa; 5♂, 6♀ (TARI), Tansui (= Tamsui, 淡水), 24.VIII.1941, leg. S. Miyamoto; 2♂, 2♀ (SEHU), Yangmingshan (陽明山), 24.VI.1965, leg. S. Kimoto; 1♂ (KMNH), same data as preceding; Taitung: 3♂, 4♀ (NHMUK), 14 km W of Chihshang (池上), 900 m, 16.XI.2008, leg. L. Dembický; 1♂ (TARI), Kotosho (= Lanyu Island, 蘭嶼), 30.VII.1908, leg. T. Shiraki; 1♂, 1♀ (TARI), same locality, 10.III.-14.IV.1920, leg. J. Sonan & T. Okuni; 2♂, 1♀ (TARI), same island, 22-26.III.1998, leg. C.-F. Lee; 1♂, 2♀ (NHMUK), same island, Yung Hsin Farm (永興農場), 29-30.VII.2008, leg. H. Mendel & M. V. L. Barclay; 1♂ (TARI), same island, 29.III.2009, leg. U. Ong; 5♂ (TARI), same island, 18.III.2012, leg. J.-C. Chen; Taoyuan: 3♂, 11♀ (TARI), Fuhsing (復興), 500 m, 6.V.1983, leg. K. C. Chou & C. C. Pan; **THAILAND.** 1♀ (KMNH), Chieng Dao, 15.VI.1965, leg. K. Morimoto.

Redescription. Male. Length 2.34–2.66 mm, width 1.20–1.33 mm. General color dark brown to blackish brown, head and prothorax yellow, legs and antennae yellow but hind femora darkened (Figs. 2A–C). Antenna (Fig. 3A) filiform, antennomeres I and II slender, III–X apically widened, ratio of length of antennomeres I–XI 1.0 : 0.6 : 0.5 : 0.5 : 0.5 : 0.5 : 0.6 : 0.6 : 0.6 : 0.6 : 0.9, ratio of length to width from antennomeres I–XI 2.7 : 1.9 : 2.9 : 2.8 : 2.2 : 1.9 : 2.0 : 1.9 : 2.0 : 2.0 : 2.9. Pronotum 1.63–1.64 times wider than long; lateral margins slightly rounded, anterolateral angles separated from lateral margins by weak emarginations,

apical margin slightly concave, basal margin moderately convex; disc smooth, lacking punctures. Elytra 1.33–1.37 times longer than wide; lateral margins rounded, widest at basal 1/4, apex convergent; dorsoventrally slightly convex, apex visible in dorsal view; disc with longitudinal lines of coarse punctures; with fine punctures between longitudinal lines. Tarsomeres I of the front and middle legs are swollen. Aedeagus (Figs. 3C–D) elongate, 4.1 times longer than wide; parallel-sided, subapically narrow, apex pointed; moderately curved in lateral view.

Female. Length 2.62–2.69 mm, width 1.28–1.33 mm. Antennae similar to those of males, ratio of length of antennomeres I–XI (Fig. 3B) 1.0 : 0.6 : 0.6 : 0.6 : 0.6 : 0.6 : 0.7 : 0.7 : 0.7 : 0.7 : 1.0; ratio of length to width from antennomere I–XI 3.3 : 2.3 : 2.7 : 2.4 : 2.5 : 2.1 : 2.1 : 2.0 : 2.0 : 2.0 : 3.1. Elytra 1.40–1.45 times longer than wide; lateral margins rounded, widest at basal 1/4, apex convergent; dorsoventrally slightly convex, apex visible in dorsal view; disc with longitudinal lines of coarse punctures; with fine punctures between longitudinal lines. Gonocoxae (Fig. 3F) are extremely slender, separated; each gonocoxa has eight long setae from apical 1/4 to apex, subapically slightly curved. Ventrite VIII (Fig. 3E) is weakly sclerotized apically, with a narrow apical area dense short setae along the apical margin, and some long setae arranged into a transverse row in lateral areas near the apical margin; the spiculum is extremely elongate. Spermathecal receptaculum (Fig. 3G) is slightly swollen, with transverse wrinkles at the basal half; the pump is slender and curved; the sclerotized spermathecal canal is short before the base of the spermathecal gland.

Color variation. Some populations from Taiwan and Myanmar have entirely yellow bodies (Figs. 2D–F). The Taiwanese populations were described as *S. fulva* Kimoto, 1996, based on this color variation. Yellow populations in Taiwan are allopatric from bicolored populations, which have been recorded from Chuanfanshih (船帆石), Lungkeng (龍坑), Oluanpi (鵝鑾鼻), and Yehliu (野柳) along the seashore (Fig. 4).

Diagnosis. The variable color patterns are not diagnostic for adults of *S. obscurofasciata* since this character is variable. This species can be identified by the apically tapering and pointed apex of the aedeagus, which is moderately curved in lateral view (Figs. 3C–D).

Food Plants. Malvaceae: *Hibiscus tiliaceus* L. (黃槿) (Figs. 5A–B), *H. taiwanensis* S. Y. Hu (山芙蓉) (Fig. 5E), *Malvaviscus arboreus* Cav. (南美朱槿) (Figs. 5C–D). In China, adults feed on *H. syriacus* L. (木槿) and *H. mutabilis* L. (木芙蓉) (Yu *et al*. 1996). Adults do not feed on *Hibiscus rosa-sinensis* L. (朱槿) even though they have been observed feeding on *Malvaviscus arboreus* located nearby (personal observation).

Taxonomic notes. *S. fulva* Kimoto, 1996 is proposed as a junior synonym of *S. obscurofasciata* based on their similar aedeagi and their shared food plant, *H. tiliaceus* L. (Fig. 5B).

Biological notes. Adults have been observed feeding on leaves of host plants. They occur in the lowlands year-round, but only a few are present at high elevations during the warmest season. The species co-occurs with *Nisotra gemella* (Erichson, 1834), also feeding on *H. tiliaceus* and *N. nigripes* Jacoby, 1894, feeding on *H. taiwanensis* (Fig. 5F), based on the field observations.

Distribution. China, Myanmar, Laos, Vietnam, Thailand, India, Sri Lanka, Taiwan, including Kinmen Island (new record), Lieyu Island. (new record), Ludao Island and Lanyu Island. Adults are widespread in the lowlands of Taiwan (Fig. 4). The new species, *S. kimotoi*, sp. nov., is confused with *S. obscurofasciata* in Thailand. Records from Vietnam and India need further confirmation since no voucher specimens were studied.

Sinocrepis kimotoi, sp. nov.

S. obscurofasciata: Kimoto 2000: 272 (Thailand). (misidentification!)

Types. Holotype ♂ (KMNH) (Figs. 1F–G). **THAILAND.** Fang, 14.VI.1965, leg. K. Morimoto. Paratype. **THAILAND.** 1♂ (KMNH). Khao Chong nr Trung, 25.VI.1965, leg. K. Morimoto. Both types were misidentified as *S. obscurofasciata* by Kimoto (2000).

Description. Male. Length 2.73 mm, width 1.42 mm. General color blackish brown, elytra black, head and prothorax yellow, legs and antennae blackish brown, but three basal antennomeres paler (Fig. 1F). Antenna (Fig. 6A) filiform, antennomeres I and II slender, III–X apically widened, ratio of length of antennomeres I–XI 1.0 : 0.6 : 0.5 : 0.5 : 0.6 : 0.6 : 0.8 : 0.8 : 0.7 : 0.7 : 1.1, ratio of length to width from antennomeres I–XI 2.5 : 2.0 : 2.3 : 1.9 : 2.2 : 2.0 : 2.4 : 2.3 : 2.2 : 2.1 : 3.4. Pronotum 1.47 times wider than long; lateral margins slightly rounded, anterolateral angles separated from lateral margins by weak emarginations, apical margin slightly concave, basal margin moderately convex; disc smooth, lacking punctures. Elytra 1.31 times longer than wide; lateral margins rounded, widest at basal 1/4, apex convergent; dorsoventrally slightly convex, apex visible in dorsal view; disc with longitudinal lines of coarse punctures; with fine punctures between longitudinal lines. Tarsomeres I of front and middle legs swollen. Aedeagus (Figs. 6B–C) elongate, 4.3 times longer than wide; parallel-sided, subapically widened, apex widely rounded with truncate process at middle; slightly curved in lateral view, apex curved.

Diagnosis. Adults of *S. kimotoi*, sp. nov. can be separated from those of *S. obscurofasciata* by the blackish-brown antennae and legs, the subapically widened aedeagus, which is straight in lateral view and curved apically.

Food Plants. Unknown.

Etymology. This new species is named after the late Dr. Shinsaku Kimoto, a chrysomelid taxonomist active from the 1960s to 2000s. He described more than two hundred new species and reported numerous new records from Taiwan.

Distribution. Thailand.

DISCUSSION

The distribution of *S. obscurofasciata* corresponds with that of its food plants. Adults of *S. obscurofasciata* are frequently found around human habitations because *Malvaviscus arboreus* is an introduced ornamental tree. The species is also common in mountainous areas where another food plant, *H. taiwanensis*, occurs. Moreover, they can survive on small islands (Fig. 4) provided that suitable food plants are present. *H. tiliaceus* (黃槿) is a coastal species utilized by *S. obscurofasciata*, which has been recorded from Lanyu Island (蘭嶼), Ludao Island (綠島), Lieyu Island (烈嶼), and Kinmen Island (金門島). Another alticite beetle, *Nisotra gemella* (Lee *et al*. 2024), also feeds on *H. tiliaceus* (黃槿), which has been recorded from Kinmen Island (金門島) and Beigan Island (北竿島). Among Taiwanese leaf beetles, *Phola octodecimguttata* (Fabricius, 1775) is recorded from many small islands of Taiwan, including Penghu Island (澎湖), Dongji Island (東吉嶼), Lanyu Island (蘭嶼), Liuchiu Island (琉球嶼), Lieyu Island (烈嶼), Dadan Island (大膽島), Kinmen Island (金門島), and Beigan Island (北竿島) due to the occurrence of one of its host plants, *Vitex rotundifolia* (海埔姜), a coastal species (Lee & Geiser 2023). Now, *Phola octodecimguttata* is also found at the Ludao Island (綠島) (unpublished data). Presumably, *S. obscurofasciata* will be recorded from more islands if extensive and continuous collection can be conducted.

The results indicate that specimens should be studied more carefully since adults of *S. kimotoi*, sp. nov., have been confused with those of *S. obscurofasciata*. Examination of aedeagi is crucial for species identification in the morphology-based taxonomic studies of Chrysomelidae (Lee *et al*. 2010; Lee 2015, 2016). Records of *S. obscurofasciata* from Myanmar, China, Thailand, Laos, Sri Lanka, and Taiwan were confirmed by examination of aedeagi. Records from other countries, such as India and Vietnam, still need further investigation.

ACKNOWLEDGEMENTS

We are grateful to the Taiwan Chrysomelid Research Team (TCRT) and citizen scientists, including Jung-Chang Chen (陳 榮 章), Hsing-Tzung Cheng (鄭興宗), Hua-Te Fang (方華德), Mei-Hua Tsou (曹美華), Ui-Ka Ong (王惟加), Tai-Chuan Wang (王泰權), and Su-Fang Yu (余素芳) for assistance in collecting material. We thank Ta-Hsiang Lee (李大翔), and Hsing-Tzung Cheng (鄭興宗) for photos of specimens, Jung-Chang Chen (陳榮章), Hsueh Lee (李雪), and Mei-Hua Tsou (曹美華) for field photography, and Chih-Kai Yang (楊智凱) for identification of host plants. We especially thank Christophe Rivier for taking photos of the syntype of *Sinocrepis micans* Chen, and Roberto Poggi for syntypes of *Crepidodera obscurofasciata* Jacoby. We thank Chang-Chin Chen (陳常卿) for assisting our study in various ways. We thank Chris Carlton for reading the draft and editing for American English style. This study was supported by the National Science and Technology Council NSTC 112-2313-B-055-001-MY3.

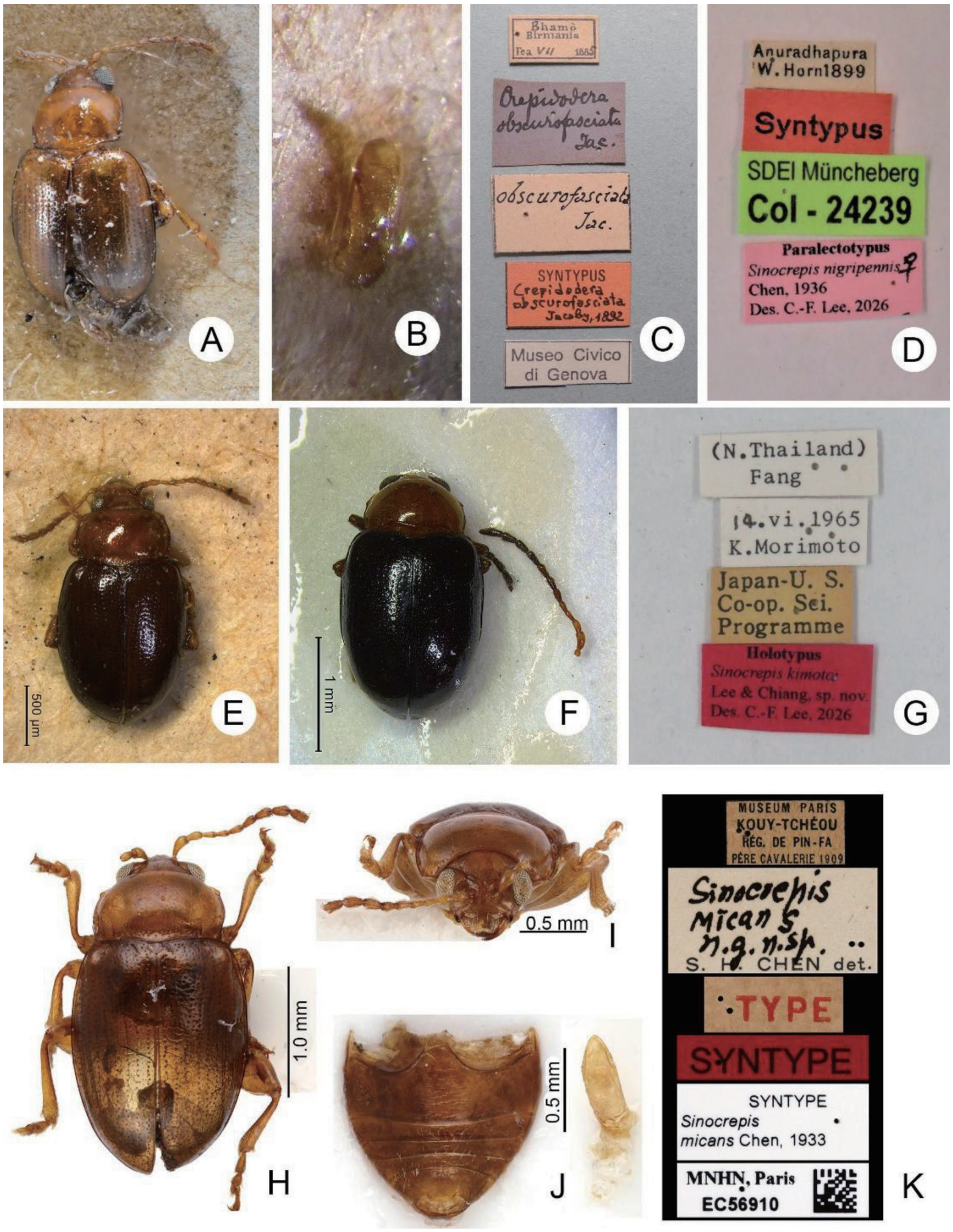

Fig. 1. Type specimens and labels of *Sinocrepis obscurofasciata*, its synonyms, and *S. kimotoi* sp. nov. A–C: *Crepidodera obscurofasciata*, A. lectotype, dorsal view; B. aedeagus, dorsal view; C. labels; D–E: *S. nigripennis*, D. labels; E. paralectotype, dorsal view; F–G: *S. kimotoi* sp. nov., F. holotype, dorsal view; G. labels; H–K: *Sinocrepis micans*, H. lectotype, dorsal view; I. ditto, front view; J. abdomen and aedeagus; K. labels.

Fig. 2. Color variations of *Sinocrepis obscurofasciata*. A. typical color pattern, dorsal view; B. ditto, ventral view; C. ditto, lateral view; D. color variation identified as *Sinocrepis fulva*, dorsal view; E. ditto, ventral view; F. ditto, lateral view.

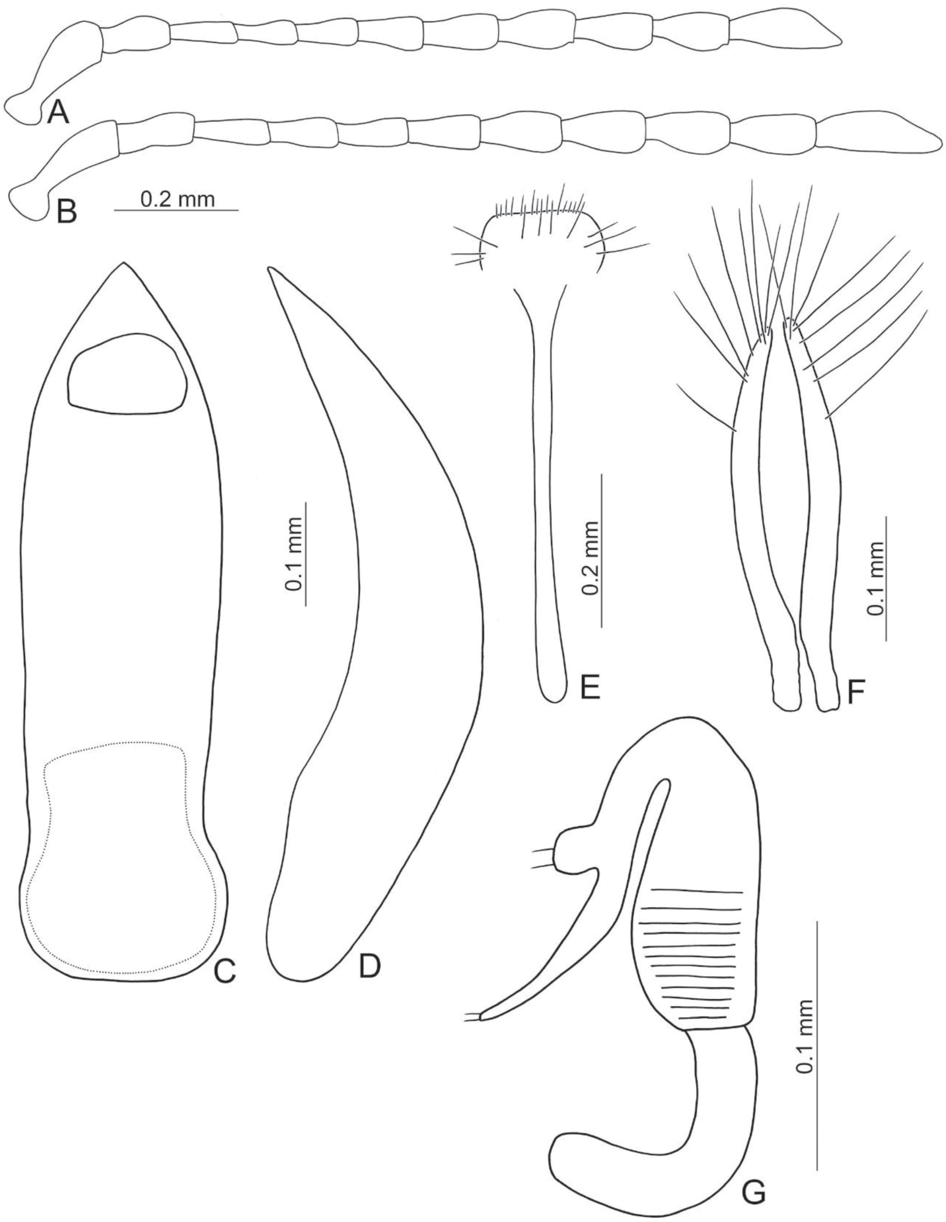

Fig. 3. Diagnostic characters of *Sinocrepis obscurofasciata*. A. antenna, male; B. antenna, female; C. aedeagus, dorsal view; D. ditto, lateral view; E. abdominal ventrite VIII, female; F. gonocoxae; G. spermatheca.

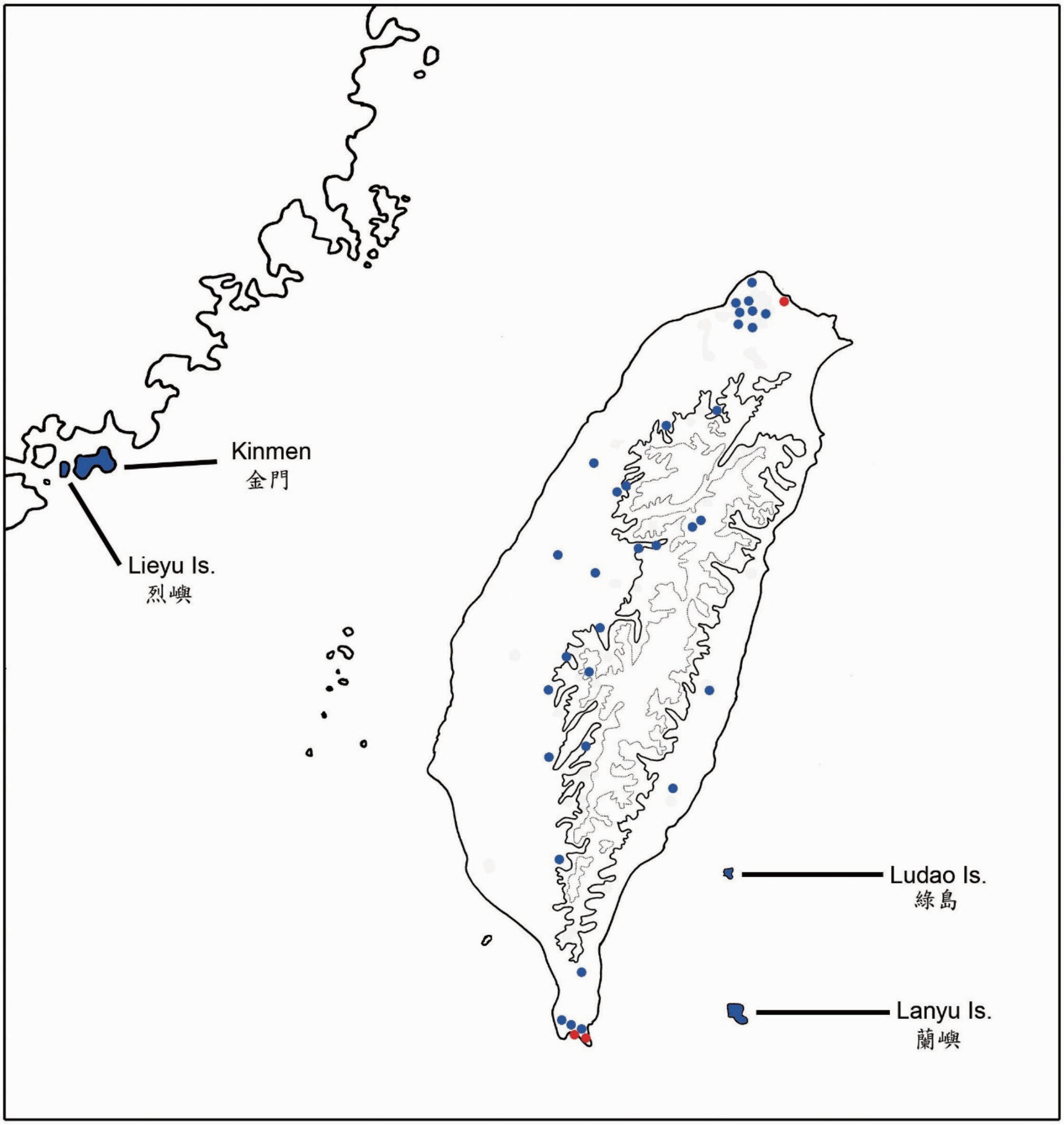

Fig. 4. Distribution map of *Sinocrepis obscurofasciata* in Taiwan, marked with red and blue circles; topographic contour lines: black: 1,000 m, gray: 2,000 m; color pattern: blue: typical populations, red color: yellow populations.

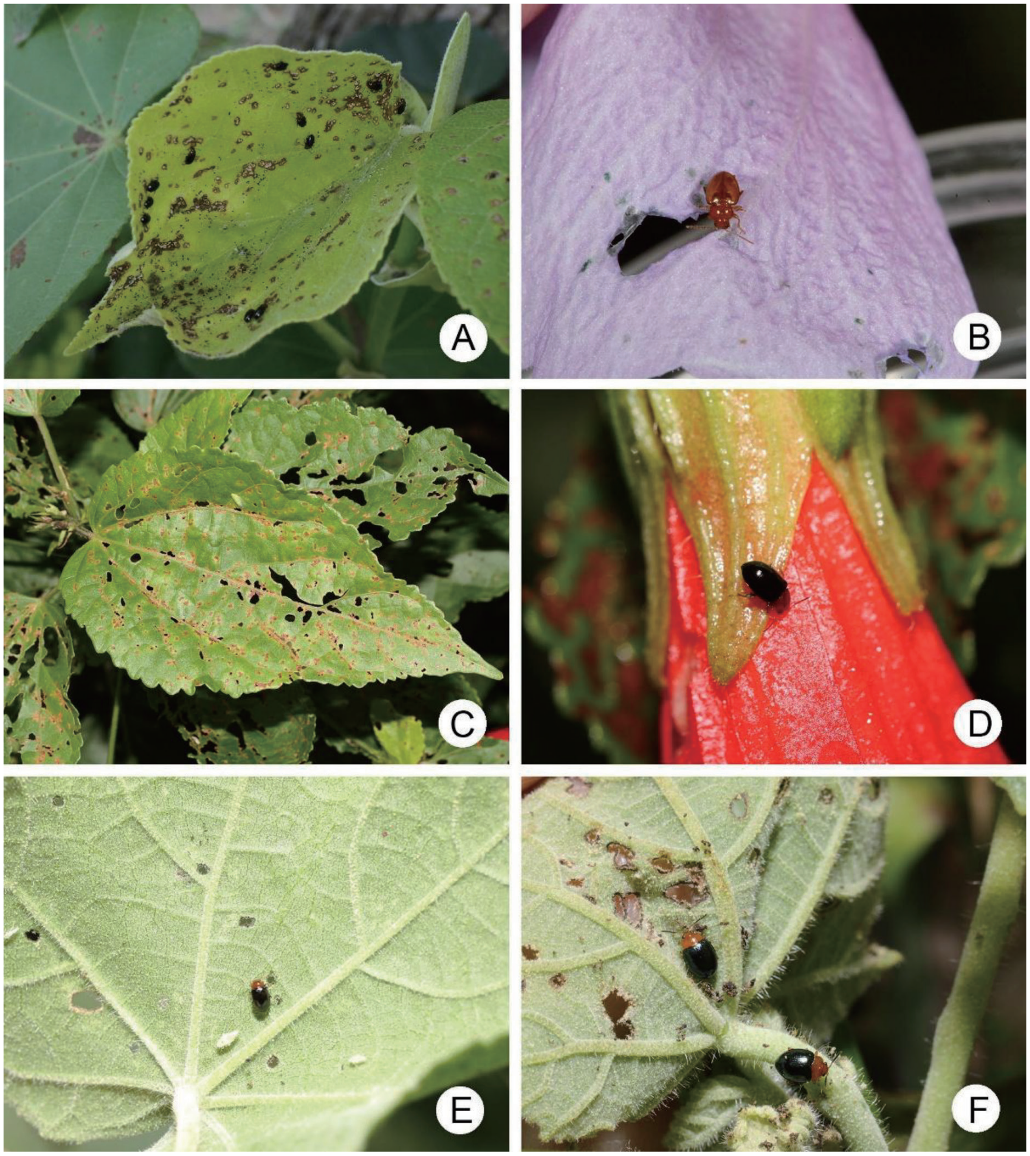

Fig. 5. Field photographs. A. several adults of *Sinocrepis obscurofasciata* feeding on leaves of *Hibiscus tiliaceus* (黃槿); B. one adult (yellow form) feeding on flower of *H. tiliaceus* (黃槿); C. feeding damage caused by adults of *S. obscurofasciata* on leaves of *Malvaviscus arboreus* (南美朱槿); D. adult of *S. obscurofasciata* feeding on flower of *M. arboreus* (南美朱槿); E. adult of *S. obscurofasciata* feeding on leaves of *H. taiwanensis* (山芙蓉); F. adults of *Nisotra nigripes* feeding on leaves of *H. taiwanensis* (山芙蓉).

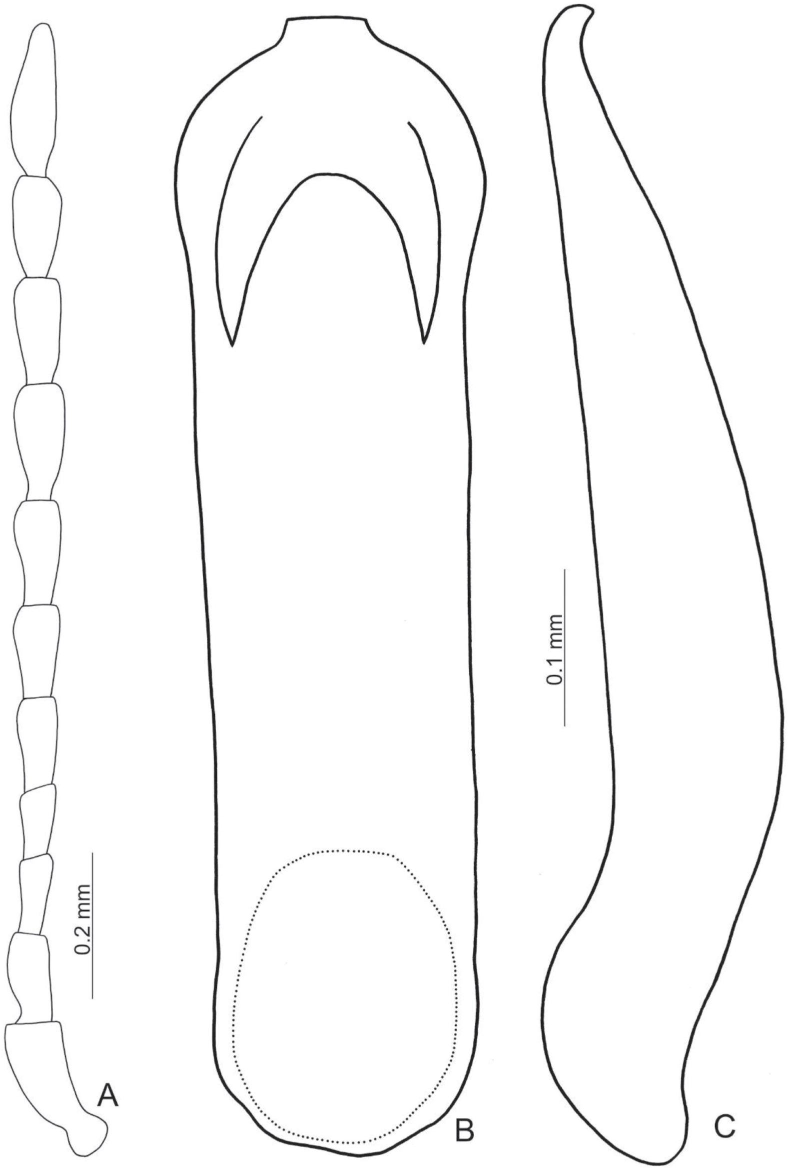

Fig. 6. Diagnostic characters of *Sinocrepis kimotoi*, sp. nov. A. antenna, male; B. aedeagus, dorsal view; C. ditto, lateral view.

REFERENCES

Chen, S. H. 1933. Study of Chinese Halticinae beetles with descriptions of some exotic new species. Sinensia 3:211–254.

Chen, S. H. 1934. Coléoptères Halticinae recueillis par M. H. Sauter a Formose. Ann. Soc. Entomol. Fr. 103:175–185. (in French)

Chen, S. H. 1936. Notes on some flea-beetles from tropical Asia (II). Sinensia 7:80–88.

Chûjô, M. 1935. Studies on the Chrysomelidae in the Japanese Empire (VIII). Subfamily Halticinae (3). Trans. Nat. Hist. Soc. Formosa 25:459–476.

Chûjô, M. 1963a. Chrysomelid-beetles from Formosa, preserved in the Hungarian Natural History Museum, Budapest. Ann. Hist.-Nat. Mus. Natl. Hung. 55:379–402.

Chûjô, M. 1963b. Chrysomelid-beetles from Formosa (Taiwan) collected by Dr. K. Baba in 1962. Niponius 2:21–30.

Duckett, C. N. and J. D. Daza. 2004. A new species of flea beetle in the genus *Alagoasa* Bechyné (Coleoptera: Chrysomelidae: Galerucinae) from highland Costa Rica. Coleopt. Bull. 58:84–96. doi:10.1649/608

Gressitt, J. L. and S. Kimoto. 1963. The Chrysomelidae (Coleopt.) of China and Korea, part 2. Pac. Ins. Mon. 1B:301–1026.

Jacoby, M. 1892. Viaggio di Leonardo Fea in Birmania e regioni vicine. LI. Description of the new genera and species of the phytophagous Coleoptera obtained by Sign. L. Fea in Burma. Ann. Mus. Civ. Stor. Nat. Genova 32:869–999.

Kimoto, S. 1966. A list of the Chrysomelid specimens of Taiwan preserved in the Zoological Museum, Berlin. Esakia 5:21–38. doi:10.5109/2351

Kimoto, S. 1970. Notes on the Chrysomelidae from Taiwan IV. Kontyû 38:205–221.

Kimoto, S. 1986. The Chrysomelidae (Insecta: Coleoptera) collected by the Nagoya University Scientific Expedition to Taiwan in 1984. Kurume Univ. J. 35:53–62.

Kimoto, S. 1987. The Chrysomelidae (Insecta: Coleoptera) collected by the Nagoya University Scientific Expedition to Taiwan in 1986. Kurume Univ. J. 36:183–194.

Kimoto, S. 1989. The Taiwanese Chrysomelidae (Insecta: Coleoptera) collected by Dr. Kintaro Baba, on the occasion of his entomological survey in 1983 and 1986. Kurume Univ. J. 38:237–272.

Kimoto, S. 1991. The Taiwanese Chrysomelidae (Insecta: Coleoptera) collected by Dr. Kintaro Baba, on the occasion of his entomological survey in 1987, 1988 and 1989. Kurume Univ. J. 40:1–27.

Kimoto, S. 1996. Notes on the Chrysomelidae from Taiwan, China, XIII. Entomol. Rev. Japan 51:27–51.

Kimoto, S. 2000. Chrysomelidae (Coleoptera) of Thailand, Cambodia, Laos and Vietnam. VII. Alticinae. Bull. Inst. Comp. Stud. Intl. Cul. Soc. 26:103–299.

Lee, C. F. 2015. The genus *Paraplotes* Laboissière, 1933 in Taiwan, a speciose group with brachyelytrous females (Coleoptera: Chrysomelidae: Galerucinae). Zootaxa 3904:223–248. doi:10.11646/zootaxa.3904.2.3

Lee, C. F. 2016. Review of the genus *Siemssenius* Weise, 1922 (Coleoptera: Chrysomelidae: Galerucinae) from Taiwan, with descriptions of five new species. Zootaxa 4158:367–384. doi:10.11646/zootaxa.4158.3.4

Lee, C. F. 2025. What drives the reduction of hindwings in Galerucinae senus stricto (Insecta, Coleoptera, Chrysomelidae)? A case study based on the Taiwanese fauna. ZooKeys 1252:111–137. doi:10.3897/zookeys.1252.142772

Lee, C. F., J. Bezděk, and C. L. Staines. 2010. A review of the genus *Agetocera* (Coleoptera: Chrysomelidae: Galerucinae) in Taiwan- Are there only two species? Zootaxa 2441:1–19. doi:10.11646/zootaxa.2441.1.1

Lee, C. F., M. Y. Chiang, M. F. Geiser, and K. H. Chuang. 2024. The genus *Nisotra* Baly, 1864 (Coleoptera, Chrysomelidae, Galerucinae, Alticini) in Taiwan, with redescriptions of four Asian species and notes on the immature stages of *N. gemella* (Erichson, 1934). ZooKeys 1205:299–331. doi:10.3897/zookeys.1205.121928

Lee, C. F. and M. F. Geiser. 2023. Taxonomic review of the genus *Phola* Weise, 1890 (Coleoptera: Chrysomelidae: Chrysomelinae). J. Taiwan Agri. Res. 72:197–218. doi:10.6156/JTAR.202309_72(3).0002

Medvedev, L. N. 2009. Alticinae of Indochina. KMK Scientific Press. Moscow, Russia. 224 pp.

Medvedev, L. N. 2015. New taxa of Chrysomelidae (Coleoptera) from Vietnam. Russian Entomol. J. 24:67–72. doi:10.15298/rusentj.24.1.06

Ruan, Y. Y. and X. K. Yang. 2017. Alticinae. p.177–223. *in*: Insect Fauna of the Qinling Mountains. Volume VII (Coleoptera III) (Yang, X. K. and R. Z. Zhang, eds.) World Publishing Corporation. Xi'an, China. 485 pp. (in Chinese)

Scherer, G. 1969. Die Alticinae des indischen Subkontinentes (Coleoptera-Chrysomelidae). Pac. Ins. Mon. 22:1–251. (in German with English abstract)

Taiwan Encyclopedia of Life (TaiEOL). n.d. Taiwan Encyclopedia of Life. https://taieol.tw/ (visit on

3/11/2026)

Yang, X. K., S. Q. Ge, R. I. Nie, Y. Y. Ruan, and W. Z. Li. 2015. Chinese Leaf Beetles. Science Press. Beijing, China. 507 pp.

Yu, P. Y., S. Y. Wang, and X. K. Yang. 1996. Economic Insect Fauna of China, Fasc. 54. Coleoptera: Chrysomeloidea (II). Science Press. Beijing, China. 324 pp. (in Chinese)

溝基葉蚤屬 (*Sinocrepis* Chen, 1933) 的分類修訂 (鞘翅目：金花蟲科：螢金花蟲亞科：葉蚤超族)

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摘要

李奇峯、江明耀。2026。溝基葉蚤屬 (*Sinocrepis* Chen, 1933) 的分類修訂 (鞘翅目：金花蟲科：螢金花蟲亞科：葉蚤超族)。台灣農業研究 75(2):201–216。

本研究重新評估溝基葉蚤屬 *Sinocrepis* Chen, 1933 所有種群名的分類地位，經由檢查模式標本及大量的標本後，本研究支持 *Sinocrepis micans* Chen, 1933 是錦葵溝基葉蚤 (*S. obscurofasciata*) 的次同物異名 (junior synonym)，而 *S. nigripennis* Chen, 1936 及 *S. fulva* Kimoto, 1996 為錦葵溝基葉蚤的新同物異名；並發表一個泰國產新種：*S. kimotoi*, sp. nov.，其模式標本是之前被錯誤鑑定為錦葵溝基葉蚤，此外本文並提供錦葵溝基葉蚤新的生物學資訊。

關鍵詞：金花蟲、錦葵科、黃槿、山芙蓉、南美朱槿。

投稿日期：2026 年 2 月 23 日；接受日期：2026 年 3 月 12 日。
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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):217–230 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0004

Research Article

Effects of Organic and Conventional Farming on Lady Beetle Diversity, Dominance, and Temporal Stability in Indian Jujube Orchards

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Abstract

Hao, H. H., Y. J. Dong, P. C. Hsu, C. F. Lee, and H. T. Shih. 2026. Effects of organic and conventional farming on lady beetle diversity, dominance, and temporal stability in Indian jujube orchards. J. Taiwan Agric. Res. 75(2):217–230.

Enhancing our understanding of how lady beetle biodiversity responds to organic and conventional farming is essential for sustainable pest management in tropical fruit systems. This study aims to compare the composition and interannual variation of lady beetle assemblages across different farming practices to assess the long-term differences in natural enemy assemblages in tropical orchards under organic and conventional management. From 2020 to 2024, lady beetle assemblages were surveyed in organically cultivated (OC) and conventionally cultivated (CC) Indian jujube orchards in Yanchao, Kaohsiung. A total of 20 species were recorded in OC orchards and 11 species in CC orchards. *Sasajiscymnus tainanensis* was the dominant species in both systems, and several species, including *Cheilomenes sexmaculata* and *Cryptogonus orbiculus*, were more abundant in OC orchards. The mycophagous species *Illeis koebelei* occurred exclusively in OC orchards. Mean species richness and total abundance per survey were significantly higher in OC orchards ($P <$ 0.05 for both metrics). Shannon diversity was significantly greater in OC orchards, whereas Berger-Parker dominance did not differ between systems. Annual total abundance was consistently higher in OC orchards, with a significant OC-CC difference detected only in 2021. Temporal variability at the community level was higher in OC orchards (coefficient of variation; CV = 0.753) than in CC orchards (CV = 0.424), indicating lower interannual stability under organic management, reflecting the natural response of the population to changes in prey resources under low pesticide disturbance. These results provide multi-year empirical evidence of distinct community patterns of lady beetles under different farming systems in subtropical fruit orchards, which can serve as a basis for ecological monitoring and planning pest management strategies.

Key words: Coccinellidae, Biological control, Predator assemblages, Agricultural intensification, Ecosystem services.

INTRODUCTION

Organic and other eco-friendly farming practices have gained increasing attention worldwide as viable strategies to enhance farmland biodiversity and support ecological functions (Reganold & Wachter 2016). Reduced pesticide input, vegetation management, and

Received: August 22, 2025; Accepted: January 28, 2026.
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habitat diversification promote the abundance and activity of natural enemies in agricultural landscapes, thereby strengthening ecosystem services such as biological control (Heimpel & Mills 2017). In Taiwan, the promotion of organic agriculture and the development of biodiversity-friendly farming systems have further highlighted the importance of multi-year ecological monitoring and indicator species for evaluating agroecosystem health.

Lady beetles (Coleoptera: Coccinellidae) are key predatory natural enemies in agricultural ecosystems, significantly contributing to the suppression of soft-bodied pests, including aphids, whiteflies, and scale insects. In perennial orchard systems, these predators help stabilize pest populations and reduce reliance on chemical insecticides, thereby supporting sustainable production (Obrycki & Kring 1998; Koch 2003; Tscharntke *et al*. 2005). Studies in temperate regions have demonstrated higher arthropod diversity and abundance under organic management compared to conventional systems (Wyss *et al*. 1995; Bengtsson *et al*. 2005; Tuck *et al*. 2014). In contrast, relatively little is known about the multi-year dynamics of lady beetle communities in tropical fruit orchards, particularly in Southeast Asia, where regional research remains limited and often short-term (Obrycki & Kring 1998; Koch 2003). In Taiwan, research has likewise concentrated on annual crops such as rice, with limited attention to predator assemblages in tropical and subtropical fruit orchards (Dong *et al*. 2023). Field surveys have identified several dominant species in local agroecosystems, including *Sasajiscymnus tainanensis* (Ohta, 1929) and *Illeis koebelei* Timberlake, 1943, reflecting the unique community structure of subtropical orchard systems (Sung *et al*. 2010; Hsu & Tsai 2024). These findings highlight the need for multi-year, system-level comparisons to understand how farming practices shape predator assemblages and their ecological functions in tropical perennial crops.

The Indian jujube (*Ziziphus mauritiana* Lam.) is a significant tropical fruit crop in Southern Taiwan, with Kaohsiung Yanchao District being a major production area (Saran *et al*. 2006; Awasthi & More 2009; Razi *et al*. 2013; Guo *et al*. 2022). These orchards are managed under either organic or conventional farming systems, which differ markedly in pesticide use, vegetation management, and habitat complexity. International research suggests that reduced pesticide inputs and more diverse ground vegetation in organic orchards can enhance natural enemy diversity and stability (Miñarro & Prida 2013; Porcel *et al*. 2018; Muneret *et al*. 2019); however, comparable evidence from tropical orchard systems remains scarce. This knowledge gap is particularly evident for the multi-year responses of lady beetle communities to different management practices- including changes in species composition, dominance structure, and temporal stability. Clarifying these patterns is essential for improving ecological management strategies, optimizing the conservation of natural enemies, and supporting sustainable fruit production in Taiwan's tropical orchard systems. We hypothesize that organic orchards support higher lady beetle diversity, more complex assemblages, distinct dominance patterns, and different temporal stability than conventionally managed orchards. Accordingly, this study conducts multi-year comparisons of Coccinellidae communities in organically cultivated (OC) and conventionally cultivated (CC) Indian jujube orchards to assess how farming practices shape predator assemblages, their community dynamics and temporal patterns, and their ecological functions.

This study aims to address this knowledge gap by conducting a multi-year ecological survey of lady beetle assemblages in organic and conventional Indian jujube orchards in Kaohsiung, Southern Taiwan. We compared species richness, abundance, dominant species structure, and temporal variation between management types to evaluate how farming practices influence predator communities. The findings provide multi-year empirical evidence for how organic management shapes predator diversity and temporal community patterns in tropical orchard systems and offer

insights for integrating biodiversity conservation into sustainable fruit production strategies in Taiwan's tropical fruit production systems.

MATERIALS AND METHODS

Study sites and experimental design

This study was conducted from 2020 to 2024 in Indian jujube (*Z. mauritiana* Lam.) orchards located in Yanchao District, Kaohsiung, Southern Taiwan. Four organically managed orchards (OC) and four conventionally managed orchards (CC) were selected for comparison. All orchards were planted with the same commercial cultivar ('Honey jujube'), and each study block was larger than 0.2 ha.

The OC orchards were located in Guanshui and Andong Sections (22.771424°N, 120.335748°E; 22.773791°N, 120.334799°E; 22.774300°N, 120.334652°E; 22.780247°N, 120.328120°E), while the CC orchards were situated in adjacent areas of Guanshui Section (22.770677°N, 120.335624°E; 22.770298°N, 120.335828°E; 22.770458°N, 120.335381°E; 22.780712°N, 120.325730°E). Selecting nearby orchards minimized differences in climate, soil, and landscape features. Kaohsiung has a tropical monsoon climate typical of Southern Taiwan, generally characterized by hot, wet summers and drier winters. Organic management followed the Organic Agriculture Promotion Act, which prohibits the use of synthetic fertilizers, chemical pesticides, genetically modified organisms (GMOs), and their by-products. In contrast, conventional orchards followed standard regional practices, including the application of chemical fertilizers and pesticides for pest and crop management.

Insect sampling and field collection

Lady beetle surveys were conducted during four crop growth stages- flowering, early fruiting, mid-fruiting, and harvest- and sampling was repeated throughout each growing season from 2020 to 2024. Each sampling date was treated as a separate sampling event for analysis.

In each orchard, four sampling subplots were established, and one yellow sticky trap (33 cm × 28 cm) was deployed on one randomly selected tree within each subplot, at each sampling event, resulting in four traps per orchard per sampling occasion. Trap installation followed standardized procedures described by Dong *et al*. (2023), with traps mounted 1.2–1.3 m above ground on black grid frames (36 cm × 28 cm), either attached to trunks or suspended from branches.

Traps were deployed for ~14 d before collection and replacement. Samples were transported to the laboratory, where lady beetles were sorted, identified to species, and counted. All records were entered into standardized datasheets to ensure consistency across sampling dates and orchards. All recorded species belonged to the family Coccinellidae, including both predatory and mycophagous species, and functional group classification followed established descriptions of feeding traits reported in regional and international literature (Sung *et al*. 2010; Hsu & Tsai 2024).

Orchard management and tree characteristics

The surveyed orchards had established production histories under either conventional or organic management. All orchards were maintained by experienced local growers following consistent annual cultivation practices. Trees selected for sampling were mature (> 10 years old), regularly pruned, and maintained with consistent annual fruit production. All trees were planted at a spacing of 6 m × 6 m and grown inside nylon mesh houses commonly used for commercial Indian jujube production in the region, primarily for fruit protection and pest exclusion. Only trees with similar vigor and canopy structure were included based on visual assessment to ensure consistent sampling conditions across orchards. Management practices in both systems followed standard local cultivation routines appropriate for Indian jujube production, including routine pruning, fertilization, irrigation, and pest management.

Statistical analyses

Statistical analyses were conducted using Python 3.10 and R 4.3.2. Analyses in Python used the SciPy, statsmodels, and pandas packages, whereas analyses conducted in R employed the vegan and car packages. The orchard (n = 4 per management type) was used as the statistical replicate. Data were aggregated at the orchard level for all analyses to avoid pseudoreplication and ensure independence among replicates. Nonparametric tests were used because the abundance and diversity variables violated the normality and equal-variance assumptions (Shapiro-Wilk and Levene's tests, $P < 0.05$). To quantify interannual stability, we compared the magnitude of year-to-year fluctuations in each response variable between organic and conventional orchards. Temporal variability (i.e., lower variance over time) is commonly interpreted as greater ecological stability. Therefore, Levene's test was applied to evaluate whether interannual variances differed between farming systems (Ives & Carpenter 2007); lower interannual variance indicates greater temporal stability in predator populations. Thus, this test directly evaluates whether one farming system supports more stable year-to-year predator dynamics. Because most variables did not meet normality assumptions ($P < 0.05$), comparisons between organic and conventional orchards were performed using the Mann-Whitney U test.

Annual comparisons between organic (OC) and conventional (CC) orchards involved only one independent test per year (OC vs CC), and therefore false discovery rate (FDR) correction was not applied. Applying FDR to small and independent two-group samples (n = 4 orchards per management type) would substantially reduce statistical power and is not recommended for independent annual contrasts. Unadjusted P-values were therefore retained for the OC-CC comparisons following standard practice for independent annual tests in ecological field studies.

To assess species abundance structure within each farming system, we compared abundance patterns across species using the Kruskal-Wallis H test. When the overall test was significant ($P < 0.05$), post hoc pairwise comparisons were conducted using Mann-Whitney U tests. In this analysis, multiple pairwise comparisons among species were performed following a significant Kruskal-Wallis test. Because these comparisons originate from the same dataset and involve repeated testing across species, the probability of type I error increases. Therefore, the Benjamini-Hochberg FDR procedure was applied to control for inflated false-positive rates while maintaining reasonable statistical power in multi-species comparisons. P-values were adjusted using the Benjamini-Hochberg FDR procedure to control the type I error rate under multiple testing.

To compare mean species richness and total abundance between organic (OC) and conventional (CC) orchards, we used the Mann-Whitney U test because the variables did not meet normality assumptions. For dominant-species responses, we compared the five most abundant species identified separately for OC and CC using Mann-Whitney U tests to evaluate species-specific differences between organic and conventional orchards.

Diversity, dominance, and stability metrics included Shannon diversity (H'), Berger-Parker dominance (d), and the coefficient of variation (CV). Differences in H' and d between farming systems were analyzed using Mann-Whitney U tests. Temporal variability was quantified using the CV for both the total community and dominant species, with annual mean abundance and CV calculated for each orchard. The CV is widely used as an index of interannual stability, with lower CV values indicating more stable year-to-year population dynamics (Wolda 1988). Differences in interannual variability between OC and CC were examined using Levene's test, which evaluates variance differences independent of mean effects.

All tests were two-sided, and statistical significance was set at $P < 0.05$. Replication at the orchard level was necessarily limited by

field logistics (n = 4 per farming system), a constraint that is common in long-term ecological monitoring of perennial orchard systems. To address this limitation, inference in this study relied on repeated observations across multiple years, with the five-year sampling framework (2020–2024) providing substantial temporal replication to support comparisons between farming systems.

Because this study was based on fixed, long-term monitoring sites rather than experimentally replicated plots, a formal a priori power analysis was not feasible. Nevertheless, the multi-year temporal design increased the robustness of statistical comparisons and allowed consistent management-related patterns to be evaluated across years, despite modest spatial replication.

RESULTS

Species composition and dominant species

From 2020 to 2024, a total of 20 lady beetle species were recorded in organic (OC) Indian jujube orchards and 11 species in conventional (CC) orchards (Tables 1 and 2). Among these, 9 species were exclusive to OC, and 2 to CC. *Sa. tainanensis* (Ohta, 1929) was the dominant species in both systems, with consistently higher mean per-survey abundance in OC orchards. Furthermore, other frequently encountered species included the predatory species *Cryptogonus orbiculus* (Gyllenhal, 1808) and *Cheilomenes sexmaculata* (Fabricius, 1781),

Table 1. Lady beetle species and their mean abundance in organic Indian jujube orchards from 2020 to 2024 (ranked by abundance).

Scientific name	Trophic guild	Mean abundance (mean ± *SE*)[z]
Sasajiscymnus tainanensis (Ohta, 1929)	Predatory	5.12 ± 1.38 a
Cryptogonus orbiculus (Gyllenhal, 1808)	Predatory	2.19 ± 0.46 b
Cheilomenes sexmaculata (Fabricius, 1781)	Predatory	1.45 ± 0.50 c
Illeis koebelei Timberlake, 1943	Mycophagous	0.27 ± 0.14 d
Sasajiscymnus fuscus (Yang, 1971)	Predatory	0.06 ± 0.03 d
Scymnus quadrillum Motschulsky, 1858	Predatory	0.05 ± 0.03 d
Scymnus ovimaculatus Sasaji, 1968	Predatory	0.04 ± 0.02 d
Jauravia limbata Motschulsky, 1858	Predatory	0.04 ± 0.02 d
Axinoscymnus nigripennis Kamiya, 1965	Predatory	0.03 ± 0.02 d
Propylea japonica (Thunberg, 1781)	Predatory	0.03 ± 0.02 d
Sasajiscymnus parenthesis (Weise, 1923)	Predatory	0.03 ± 0.02 d
Sticholotis taiwanensis Miyatake, 1982	Predatory	0.03 ± 0.02 d
Scymnus yangi Yu et Pang, 1993	Predatory	0.01 ± 0.01 d
Lemnia saucia (Mulsant, 1850)	Predatory	0.01 ± 0.01 d
Lemnia biplagiata (Swartz, 1808)	Predatory	0.01 ± 0.01 d
Sticholotis formosana Weise, 1923	Predatory	0.01 ± 0.01 d
Rodolia okinawensis Miyatake, 1959	Predatory	0.01 ± 0.01 d
Platynaspidius maculosus (Weise, 1910)	Predatory	0.01 ± 0.01 d
Scymnus hoffmanni Weise, 1879	Predatory	0.01 ± 0.01 d
Platynaspidius quinquepunctatus Miyatake, 1961	Predatory	0.01 ± 0.01 d

[z] Differences in species abundance were tested using Kruskal-Wallis with post hoc Mann-Whitney U tests (false discovery rate (FDR)-adjusted). Species sharing the same letter are not significantly different at $P < 0.05$.

both of which were more abundant in OC orchards (Table 3). The mycophagous species *I. koebelei* Timberlake, 1943, occurred exclusively in OC orchards. *Cr. ohtai* Sasaji, 1968, was detected only in CC orchards. Species-level abundances varied significantly among species within each farming system as indicated by Kruskal-Wallis tests (OC: H = 850.04, P < 0.0001; CC: H = 210.71, P < 0.0001).

Species richness, abundance, and diversity indices

Mean species richness per survey was significantly higher in OC orchards (1.94 ± 0.11 species) than in CC orchards (1.43 ± 0.10 species) (Mann-Whitney U = 3,168.5, P = 0.0033). Total abundance per survey was also higher in OC (11.37 ± 1.76 individuals) than in CC orchards (4.49 ± 0.60 individuals) (U = 3,585.0, P < 0.0001) (Table 4).

Shannon diversity (H') was significantly greater in OC orchards (0.385 ± 0.043) than in CC orchards (0.240 ± 0.052) (U = 3,018.5, P = 0.0243). Berger-Parker dominance (d) did not differ significantly between farming systems (U = 2,059.5, P = 0.0567), reflecting the consistently high relative abundance of the dominant predator *Sa. tainanensis* in both orchard types (Table 5). Annual patterns of Shannon diversity are presented in Fig. 1.

Table 2. Lady beetle species and their mean abundance in conventional Indian jujube orchards from 2020 to 2024 (ranked by abundance).

Scientific name	Trophic guild	Mean abundance (mean ± SE)[z]
Sasajiscymnus tainanensis (Ohta, 1929)	Predatory	1.78 ± 0.52 a
Cheilomenes sexmaculata (Fabricius, 1781)	Predatory	0.90 ± 0.28 b
Propylea japonica (Thunberg, 1781)	Predatory	0.08 ± 0.04 c
Cryptogonus ohtai Sasaji, 1968	Predatory	0.06 ± 0.03 c
Cryptogonus orbiculus (Gyllenhal, 1808)	Predatory	0.06 ± 0.03 c
Scymnus quadrillum Motschulsky, 1858	Predatory	0.04 ± 0.00 c
Sasajiscymnus fuscus (Yang, 1971)	Predatory	0.04 ± 0.03 c
Axinoscymnus nigripennis Kamiya, 1965	Predatory	0.04 ± 0.03 c
Rodolia okinawensis Miyatake, 1959	Predatory	0.02 ± 0.02 c
Olla v-nigrum (Mulsant, 1866)	Predatory	0.02 ± 0.02 c
Rodolia cardinalis (Mulsant, 1850)	Predatory	0.02 ± 0.02 c

[z] Differences in species abundance were tested using Kruskal-Wallis with post hoc Mann-Whitney U tests (false discovery rate (FDR)-adjusted). Species sharing the same letter are not significantly different at P < 0.05.

Table 3. Comparison of dominant lady beetle species between organically cultivated (OC) and conventionally cultivated (CC) jujube orchards based on mean abundance per survey.

Species	Organic[z] (mean ± SE)	Conventional (mean ± SE)	U-value	P-value
Sasajiscymnus tainanensis (Ohta, 1929)	5.12 ± 1.38	1.78 ± 0.52	3,257.5	0.0020*
Cryptogonus orbiculus (Gyllenhal, 1808)	2.19 ± 0.46	0.06 ± 0.03	3,621.0	< 0.0001*
Cheilomenes sexmaculata (Fabricius, 1781)	1.45 ± 0.50	0.90 ± 0.28	2,254.0	0.2131
Illeis koebelei Timberlake, 1943	0.27 ± 0.14	0.00 ± 0.00	2,677.5	0.0521
Sasajiscymnus fuscus (Yang, 1971)	0.06 ± 0.03	0.04 ± 0.03	2,505.0	0.9485
Propylea japonica (Thunberg, 1781)	0.03 (0.02)	0.08 ± 0.04	2,379.5	0.1939
Cryptogonus ohtai Sasaji, 1968	0.00 ± 0.00	0.06 ± 0.03	2,352.0	0.0160*

[z] Mean values represent abundance per survey (mean ± SE). Differences between OC and CC were evaluated using the Mann-Whitney U test. Asterisks (*) indicate significant differences at the P < 0.05.

Table 4. Comparison of mean species richness and total abundance of lady beetles per survey between organically cultivated (OC) and conventionally cultivated (CC) jujube orchards from 2020 to 2024.

Variable	Organic (mean ± *SE*)	Conventional (mean ± *SE*)	*U*-value	*P*-value
Mean richness (species/survey)[z]	1.94 ± 0.11	1.43 ± 0.10	3,168.5	0.0033
Mean abundance (individuals/survey)[z]	11.37 ± 1.76	4.49 ± 0.60	3,585.0	< 0.0001

[z] Mann-Whitney *U* tests were used to compare mean species richness and total abundance per survey between OC and CC jujube orchards. All comparisons were significant at $P < 0.05$.

Table 5. Comparison of lady beetle diversity (Shannon *H′*), dominance (Berger-Parker *d*), and interannual stability (coefficient of variation; CV) between organically cultivated (OC) and conventionally cultivated (CC) jujube orchards.

Index	Mean ± *SE*[z] (OC)	CV (OC)	Mean ± *SE* (CC)	CV (CC)	*U*-value	*P*-value
Shannon *H′*	0.385 ± 0.043	1.113	0.240 ± 0.052	1.558	3,018.5	0.0243
Berger-Parker *d*	0.826 ± 0.021	0.254	0.867 ± 0.029	0.238	2,059.5	0.0567

[z] Shannon diversity (*H′*) and Berger-Parker dominance (*d*) were compared between OC and CC orchards using the Mann-Whitney *U* test. Interannual stability was evaluated using the CV, which was not subjected to significance testing. Statistical significance was set at $P < 0.05$.

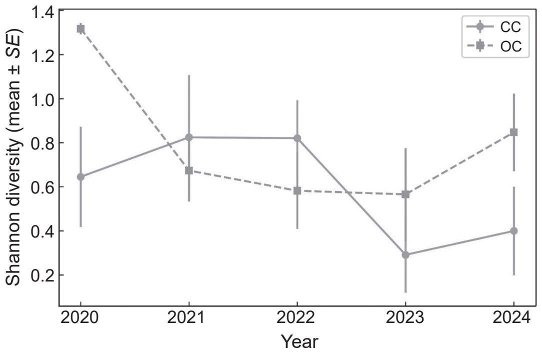

Fig. 1. Shannon diversity (mean ± *SE*) of lady beetle assemblages under organically cultivated (OC) and conventionally cultivated (CC) management in jujube orchards from 2020 to 2024. No significant differences were detected between OC and CC in any year (Mann-Whitney *U* tests; all $P \geq 0.05$).

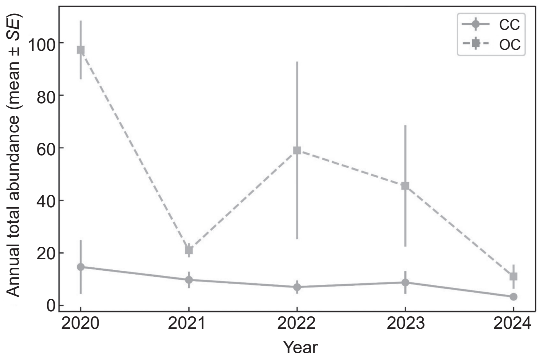

Fig. 2. Annual total abundance of lady beetles (mean ± *SE*) under organically cultivated (OC) and conventionally cultivated (CC) management in jujube orchards from 2020 to 2024. Significant differences between OC and CC were detected in 2021 ($P < 0.05$), whereas in all other years, no significant differences were observed ($P \geq 0.05$).

Annual abundance patterns

Annual total abundance of lady beetles was higher in OC orchards than in CC orchards throughout the five-year period (Fig. 2). A significant OC-CC difference was observed only in 2021 ($P < 0.05$), consistent with the annual summary in Table 4, whereas all other years showed no significant differences ($P \geq 0.05$).

Annual abundance of *Sa. tainanensis*, the dominant species, did not differ significantly between OC and CC orchards in any year (Fig. 3). *Ch. sexmaculata* exhibited a similar pattern, with no significant OC-CC differences across years and two borderline comparisons (2020 and 2024; Fig. 4). These temporal patterns correspond with the overall abundance differences summarized in Table 4.

Temporal variability and community stability

Annual total abundance from 2020 to 2024 averaged 184.80 ± 62.26 individuals per year

in OC orchards and 31.20 ± 5.91 individuals per year in CC orchards (Table 6). Interannual variance differed significantly between the two systems (Levene's stat = 6.515, $P = 0.0340$).

Community-level temporal variability (CV) was 0.753 in OC orchards and 0.424 in CC orchards (Fig. 5), indicating greater temporal instability in OC orchards compared with CC orchards. Species-level CV values for both dominant species were relatively high, indicating considerable interannual fluctuations in their populations.

For *Sa. tainanensis*, the difference in interannual variance between farming systems was marginally non-significant ($P = 0.0551$), suggesting a trend toward greater year-to-year fluctuations in OC orchards.

DISCUSSION

Community patterns under different management systems

Lady beetle assemblages in this study showed consistently higher species richness, abundance, and functional heterogeneity in organic (OC) orchards than in conventional (CC) orchards, reflecting the well-established pattern that reduced pesticide input and greater vegetation heterogeneity promote natural enemy communities in agroecosystems (Bengtsson *et al.* 2005; Tuck

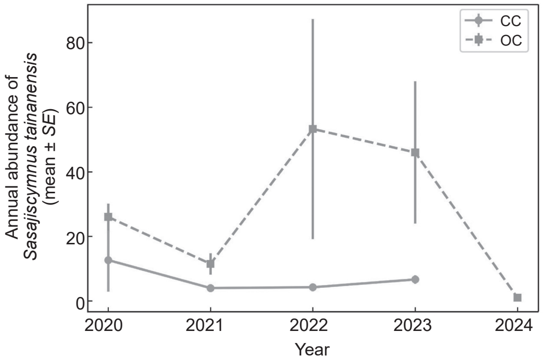

Fig. 3. Annual abundance of *Sasajiscymnus tainanensis* (Ohta, 1929) (mean ± *SE*) under organically cultivated (OC) and conventionally cultivated (CC) management in jujube orchards from 2020 to 2024. No significant differences between OC and CC were detected in any year (Mann-Whitney *U* tests; all $P \geq 0.05$).

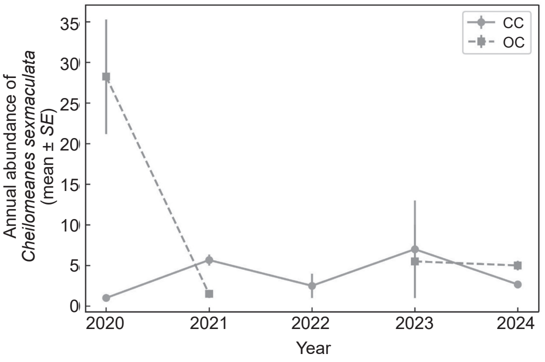

Fig. 4. Annual abundance of *Cheilomenes sexmaculata* (Fabricius, 1781) (mean ± *SE*) under organically cultivated (OC) and conventionally cultivated (CC) management in jujube orchards from 2020 to 2024. No significant differences between OC and CC were detected in any year (Mann-Whitney *U* tests; all $P \geq 0.05$). Two years (2020 and 2024) showed borderline differences (2020: $P = 0.10$; 2024: $P = 0.08$), but neither reached statistical significance.

Table 6. Comparison of mean abundance, temporal stability (coefficient of variation; CV), and interannual variance (Levene's test) of dominant lady beetle species and total community under organically cultivated (OC) and conventionally cultivated (CC) jujube orchard management.

Species/Community	Mean (*SE*)[z] (OC)	Mean (*SE*) (CC)	Levene's stat	*P*-value	CV (OC)	CV (CC)
Sasajiscymnus tainanensis (Ohta, 1929)	100.40 ± 36.71	22.75 ± 5.15	5.283	0.0551	0.817	0.453
Cheilomenes sexmaculata (Fabricius, 1781)	35.50 ± 25.95	9.20 ± 2.78	1.265	0.2977	1.462	0.676
Total (all species)	184.80 ± 62.26	31.20 ± 5.91	6.515	0.0340	0.753	0.424

[z] Annual mean abundance (mean ± *SE*) and interannual stability (CV) were calculated for dominant species and the total community from 2020 to 2024. Interannual variance between OC and CC orchards was compared using Levene's test; *P*-values shown in the table correspond to Levene's test. Interannual stability based on CV was not subjected to significance testing. Statistical significance was set at $P < 0.05$.

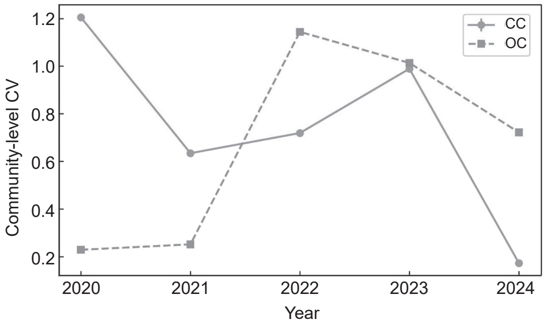

Fig. 5. Community-level temporal variability (coefficient of variation; CV) of lady beetle abundance under organically cultivated (OC) and conventionally cultivated (CC) management in jujube orchards from 2020 to 2024. No significant differences were detected between OC and CC across years ($P \geq 0.05$).

et al. 2014). The distinct species composition observed between management systems underscores this pattern. While *Sa. tainanensis* (Ohta, 1929) remained the dominant predator under both systems- indicating strong ecological adaptability and a broad prey range- several other predators, including *Cr. orbiculus* (Gyllenhal, 1808) and *Ch. sexmaculata* (Fabricius, 1781), were more abundant in OC orchards (Table 3). The exclusive occurrence of the mycophagous species *I. koebelei* Timberlake, 1943, in OC orchards further suggests that organic management supports more functionally diverse assemblages. Similar trends have been documented across Taiwanese agroecosystems, where *Sa. tainanensis* and other predatory Coccinellidae consistently respond positively to reduced pesticide disturbance (Sung *et al.* 2010; Dong *et al.* 2023; Hsu & Tsai 2024). Together, these patterns indicate that organic orchards maintain not only higher predator richness but also a broader functional spectrum within predator assemblages than conventionally managed orchards. Overall, these findings demonstrate that organic management promotes both taxonomic and functional diversification within lady beetle communities, strengthening the ecological basis for sustained natural pest suppression.

Ecological mechanisms underlying diversity and stability

Several mechanisms may explain why organic orchards supported richer, more dynamic lady beetle communities than conventional systems. Reduced pesticide disturbance is likely the primary driver, as broad-spectrum insecticides used in conventional orchards can suppress predator survival, impair reproduction, and disrupt population recovery following seasonal fluctuations (Porcel *et al.* 2018; Muneret *et al.* 2019). In contrast, organic orchards maintain higher structural heterogeneity through groundcover vegetation, flowering weeds, and leaf-litter layers, which collectively provide alternative prey, microhabitats, and refuges that sustain predator activity even during periods of low pest density. These habitat-mediated processes are consistent with the ecological principles of conservation biological control, where diverse vegetation promotes prey availability and stabilizes predator populations (Heimpel & Mills 2017).

Prey dynamics may also contribute to the observed patterns. Mobile generalist predators such as *Ch. sexmaculata* and *Cr. orbiculus* are known to rapidly track aphid and soft-scale outbreaks, a behavior well-documented for aphidophagous Coccinellidae (Obrycki & Kring 1998; Kindlmann & Dixon 1999). Such prey-tracking responses often generate strong interannual fluctuations when prey resources appear in short pulses rather than continuously, which is consistent with the higher CV observed in organic orchards. Although our field survey did not directly quantify prey populations (e.g., aphids, whiteflies, scale insects), previous work in Taiwanese rice systems provides important dietary evidence that helps explain the observed patterns. Stable-isotope analyses have revealed that predator dietary composition differs markedly between organic and conventional fields, with predators consuming a higher proportion of pest-derived prey in conventional systems and exhibiting broader prey spectra in organic systems (Hsu *et al.* 2025). These dietary shifts reflect

underlying differences in prey availability and resource heterogeneity between farming systems and may similarly influence the dynamics of lady beetle assemblages in jujube orchards. Temporal stability is ecologically important because more stable predator populations can provide more reliable and sustained biological control services in perennial fruit systems, where pest outbreaks vary substantially from year to year.

Seasonal climatic variation may further influence these patterns. Taiwan's tropical-subtropical rainfall regime produces pronounced seasonal shifts that shape insect phenology and population size (Wolda 1988). Under such conditions, reduced chemical disturbance in organic orchards likely allows predator populations to fluctuate with environmental and prey-driven cues rather than being suppressed by pesticide use, resulting in predator communities that fluctuate more strongly but maintain long-term presence and functional roles despite interannual variation.

Although the difference in interannual variance of *Sa. tainanensis* between farming systems was only marginally significant ($P = 0.0551$), this pattern suggests a biologically meaningful trend in which the dominant predator responds to reduced pesticide disturbance yet still exhibits notable year-to-year fluctuations. Such borderline significance is common in long-term ecological datasets with limited replication, and the observed effect remains consistent with the broader pattern of heightened predator activity and community dynamism in organic orchards.

Cross-system and regional comparisons

The patterns observed in this study are consistent with findings from other Taiwanese agroecosystems, where organic and low-input management typically support richer predator assemblages and more heterogeneous community structures. Similar trends have been documented in pomelo, citrus, and rice systems, in which predatory Coccinellidae- particularly *Sa. tainanensis*, *Cr. orbiculus*, and *Ch. sexmaculata*- respond positively to reduced pesticide use and increased groundcover vegetation (Sung *et al*. 2010; Hsu & Tsai 2024). These cross-crop similarities suggest that the mechanisms identified here, including prey-tracking behavior, habitat heterogeneity, and minimized chemical disturbance, operate broadly across subtropical orchard and field systems.

The patterns identified in this study are not unique to Taiwan but reflect broader ecological responses observed in diverse farming systems worldwide. Comparable outcomes have also been reported internationally. A synthesis from Brazil highlighted that predatory Coccinellidae consistently exhibit higher diversity and functional importance in low-input and heterogeneous farming systems (de Paula Malheiros *et al*. 2025). Field experiments in India have demonstrated that organic vegetable farms support significantly higher lady beetle richness and abundance compared to conventional fields, primarily due to reduced pesticide pressure and increased vegetation diversity (Jesu Rajan *et al*. 2019). Similarly, studies in Eastern Europe revealed that organic wheat, oat, and buckwheat systems support more diverse and structurally connected predatory Coccinellidae communities than conventionally managed fields (Grabovska *et al*. 2021).

These case studies align with global meta-analyses, which demonstrate that organic and low-input farming systems consistently support higher natural enemy diversity, stronger biological control potential, and more resilient predator communities (Bengtsson *et al*. 2005; Letourneau *et al*. 2009; Tuck *et al*. 2014). Although dominant species vary among regions, the ecological processes shaping predator assemblages- reduced disturbance, increased vegetation complexity, and enhanced resource heterogeneity- remain widely consistent across agroecosystems. Collectively, these cross-system comparisons indicate that the advantages of organic management for sustaining diverse and dynamic lady beetle assemblages represent a generalizable and widely observed ecological pattern across low-input farming systems.

Management and policy implications

The clearer separation of lady beetle assemblages between organic and conventional orchards provides important insights for orchard management and biodiversity-based pest regulation. The consistently higher richness and functional diversity observed in organic orchards suggest that maintaining groundcover vegetation, reducing pesticide disturbance, and allowing natural vegetation structure to develop can strengthen predator communities and, in turn, enhance biological control services. This interpretation aligns with global assessments of biodiversity-friendly farming, which consistently show that reducing chemical inputs and increasing habitat complexity promote resilient natural enemy communities across diverse agroecosystems (Bengtsson *et al*. 2005; Letourneau *et al*. 2009; Tuck *et al*. 2014).

These findings also provide practical guidance for ecological orchard management in subtropical systems. Practices such as maintaining flowering weeds within inter-rows, conserving leaf-litter layers, and minimizing broad-spectrum insecticide use during peak predator activity periods align with established principles of conservation biological control. Foundational synthesis work has shown that groundcover vegetation and habitat complexity can enhance predator foraging efficiency, survival, and persistence by supplying alternative prey, shelter, and favorable microclimates (Bugg & Waddington 1994; Landis *et al*. 2000). Empirical studies further demonstrate that native flowering plant strips can increase natural enemy abundance and activity in perennial fruit systems, highlighting the value of floral resources as part of habitat-management strategies (Walton & Isaacs 2011). Importantly, our results suggest that temporal variability in predator abundance- often interpreted as instability- should instead be viewed as a natural ecological response to prey fluctuations under conditions of low chemical disturbance. This interpretation is consistent with evidence showing that reduced pesticide inputs mitigate sublethal and behavioral disruptions to beneficial arthropods (Desneux *et al*. 2007) and support more resilient natural enemy communities at the landscape scale (Geiger *et al*. 2010). Collectively, these insights underscore the potential of organic orchards to function as reservoirs of natural enemies within mixed agricultural landscapes.

From a policy perspective, these results support Taiwan's ongoing efforts to expand organic and low-pesticide farming systems. Global syntheses have shown that organic agriculture consistently enhances biodiversity and associated ecosystem functions across a wide range of production landscapes (Bengtsson *et al*. 2005; Ponisio *et al*. 2015). By demonstrating that predator communities respond predictably and positively to reduced chemical disturbance, our study provides empirical evidence that biodiversity-oriented management can strengthen ecological stability and long-term pest regulation- a finding consistent with broader evaluations of ecosystem service provisioning in diversified farming systems (Garibaldi *et al*. 2017). These insights may help inform regional habitat management programs, certification standards, and incentive schemes designed to promote functional biodiversity and ecological resilience in fruit orchards.

Study limitations

This study focused on long-term patterns of lady beetle assemblages under contrasting farming systems but did not directly quantify prey populations, climatic variables, or detailed pesticide application records at each orchard. As a result, the mechanisms underlying observed differences in diversity, abundance, and temporal variability were inferred from community-level patterns rather than being explicitly tested. Future studies integrating concurrent monitoring of prey dynamics, local climatic conditions, and management inputs would provide stronger mechanistic insights into the drivers of predator community stability in tropical orchard systems.

CONCLUSIONS

Organic Indian jujube orchards in Southern Taiwan supported significantly higher lady beetle species richness, greater abundance, and higher interannual variability than conventional orchards. The dominant predator *Sa. tainanensis* (Ohta, 1929) responded strongly to organic management, a pattern consistent with the combined effects of reduced pesticide disturbance and increased vegetation heterogeneity. These results provide robust empirical evidence that organic practices enhance predator diversity and community resilience in subtropical orchard ecosystems. Moreover, the distinct species assemblages observed in Taiwan- compared to temperate and subtropical jujube systems abroad- highlight the importance of region-specific biodiversity baselines when evaluating the impacts of management.

In practical terms, integrating organic farming principles with habitat-enhancing actions- such as maintaining ground cover, conserving floral resources, and minimizing pesticide inputs- can simultaneously reduce chemical dependency and strengthen natural enemy communities. Embedding these strategies into Taiwan's fruit integrated pest management (IPM) guidelines and pesticide-reduction programs would promote ecological sustainability and reinforce the long-term resilience of biological control services in subtropical fruit production systems.

ACKNOWLEDGMENTS

This study was financially supported by the Ministry of Agriculture, Taiwan, under the Scientific Research Project 114-AgriSci-4.4.2-Agri-02. The authors sincerely thank the Ministry for its generous support. We also acknowledge the reviewers' constructive suggestions, which helped improve the clarity of this manuscript.

REFERENCES

Awasthi, O. P. and T. A. More. 2009. Genetic diversity and status of *Ziziphus* in India. Acta Hortic. 840:33–40. doi:10.17660/ActaHortic.2009.840.2

Bengtsson, J., J. Ahnström, and A. C. Weibull. 2005. The effects of organic agriculture on biodiversity and abundance: A meta-analysis. J. Appl. Ecol. 42:261–269. doi:10.1111/j.1365-2664.2005.01005.x

Bugg, R. L. and C. Waddington. 1994. Using cover crops to manage arthropod pests of orchards: A review. Agric. Ecosyst. Environ. 50:11–28. doi:10.1016/0167-8809(94)90121-X

de Paula Malheiros, K., M. V. de Oliveira Santana, C. H. Marchiori, and É. M. de Melo. 2025. The importance of ladybugs (Coleoptera: Coccinellidae) for agriculture and biodiversity in Brazil. Middle East Res. J. Microbiol. Biotechnol. 5:15–25. doi:10.36348/merjmb.2025.v05i01.003

Desneux, N., A. Decourtye, and J. M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52:81–106. doi:10.1146/annurev.ento.52.110405.091440

Dong, Y. J., W. H. Yang, P. C. Hsu, C. F. Lee, M. J. Tseng, J. Y. Chang, … H. T. Shih. 2023. Differences in lady beetle species composition between conventional and organic farming environments in Taiwan. J. Taiwan Agric. Res. 72:343–358. (in Chinese with English abstract) doi:10.6156/JTAR.202312_72(4).0005

Garibaldi, L. A., B. Gemmill-Herren, R. D'Annolfo, B. E. Graeub, S. A. Cunningham, and T. D. Breeze. 2017. Farming approaches for greater biodiversity, livelihoods, and food security. Trends Ecol. Evol. 32:68–80. doi:10.1016/j.tree.2016.10.001

Geiger, F., J. Bengtsson, F. Berendse, W. W. Weisser, M. Emmerson, M. B. Morales, … P. Inchausti. 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic Appl. Ecol. 11:97–105. doi:10.1016/j.baae.2009.12.001

Grabovska, T., M. Jelínek, and V. Shevchenko. 2021. Effect of organic farming on the ladybird beetle diversity (Coleoptera: Coccinellidae). Agrobìologìâ 1(163):188–197. doi:10.33245/2310-9270-2021-163-1-188-197

Guo, J. H., M. C. Hsu, K. D. Chiou, W. L. Lee, and Y. S. Liang. 2022. Studies on the physico-chemical changes in fruits of jujube (*Ziziphus mauritiana* Lam.) 'Tainung No. 9', 'Tainung No. 12' and 'Tainung No. 13' during development and storage. J. Taiwan Agric. Res. 71:209–229. (in Chinese with English abstract) doi:10.6156/JTAR.202209_71(3).0003

Heimpel, G. E. and N. J. Mills. 2017. Biological Control: Ecology and Applications. Cambridge University Press. Cambridge, UK. 380 pp.

Hsu, G. C., J. A. Ou, M. H. Ni, Z. H. Lin, and C. K. Ho. 2025. Generalist predators function as pest specialists: Examining diet composition of spiders and ladybeetles across rice crop stages. J. Appl. Ecol. 62:1889–1901. doi:10.1111/1365-2664.70066

Hsu, Y. T. and S. J. Tsai. 2024. Investigation of specific pest and ladybird beetles on atemoya orchard between different farming types in Taitung. TTDARES Bull. 34:69–88. (in Chinese with English abstract)

Ives, A. R. and S. R. Carpenter. 2007. Stability and diversity of ecosystems. Science 317(5834):58–62. doi:10.1126/science.1133258

Jesu Rajan, S., E. Sree Latha, K. Sneha Madhuri, R. Vijayaraghavendra, and C. H. Sreenivasa Rao. 2019. Predatory coccinellids diversity in organic vegetable farming systems: Conservation and mass production. J. Entomol. Zool. Stud. 7(1):1148–1151.

Kindlmann, P. and A. F. G. Dixon. 1999. Strategies of aphidophagous predators: Lessons for modelling insect predator–prey dynamics. J. Appl. Entomol. 123:397–399. doi:10.1046/j.1439-0418.1999.00390.x

Koch, R. L. 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non-target impacts. J. Insect Sci. 3:32. doi:10.1093/jis/3.1.32

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45:175–201. doi:10.1146/annurev.ento.45.1.175

Letourneau, D. K., J. A. Jedlicka, S. G. Bothwell, and C. R. Moreno. 2009. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annu. Rev. Ecol. Evol. Syst. 40:573–592. doi:10.1146/annurev.ecolsys.110308.120320

Miñarro, M. and E. Prida. 2013. Hedgerows surrounding organic apple orchards in north-west Spain: Potential to conserve beneficial insects. Agric. For. Entomol. 15:382–390. doi:10.1111/afe.12025

Muneret, L., A. Auriol, D. Thiéry, and A. Rusch. 2019. Organic farming at local and landscape scales fosters biological pest control in vineyards. Ecol. Appl. 29:e01818. doi:10.1002/eap.1818

Obrycki, J. J. and T. J. Kring. 1998. Predaceous Coccinellidae in biological control. Annu. Rev. Entomol. 43:295–321. doi:10.1146/annurev.ento.43.1.295

Ponisio, L. C., L. K. M'Gonigle, K. C. Mace, J. Palomino, P. de Valpine, and C. Kremen. 2015. Diversification practices reduce organic to conventional yield gap. Proc. Biol. Sci. 282:20141396. doi:10.1098/rspb.2014.1396

Porcel, M., G. K. S. Andersson, J. Pålsson, and M. Tasin. 2018. Organic management in apple orchards: Higher impacts on biological control than on pollination. J. Appl. Ecol. 55:2779–2789. doi:10.1111/1365-2664.13247

Razi, M. F. D., R. Anwar, S. M. A. Basra, M. M. Khan, and I. A. Khan. 2013. Morphological characterization of leaves and fruit of jujube (*Ziziphus mauritiana* Lamk.) germplasm in Faisalabad, Pakistan. Pak. J. Agric. Sci. 50:211–216.

Reganold, J. P. and J. M. Wachter. 2016. Organic agriculture in the twenty-first century. Nat. Plants 2:15221. doi:10.1038/nplants.2015.221

Saran, P. L., A. K. Godara, I. S. Yadav, S. K. Sehrawat, and G. Lal. 2006. Morphological diversity among Indian jujube (*Ziziphus mauritiana* Lamk.) genotypes collected at Hisar, India. J. Food Agric. Environ. 4(2):172–175. doi:10.1234/4.2006.868

Sung, I. H., Y. F. Wu, M. Y. Lin, and S. K. Chen. 2010. Seasonal occurrence, hosts, life history and the biocontrol potential of a mycophagus ladybird *Illeis koebelei* (Coleoptera: Coccinellidae: Halyziini) in Southern Taiwan. Formosan Entomol. 30:247–261. (in Chinese with English abstract) doi:10.6661/TESFE.2010019

Tscharntke, T., A. M. Klein, A. Kruess, I. Steffan-Dewenter, and C. Thies. 2005. Landscape perspectives on agricultural intensification and biodiversity-Ecosystem service management. Ecol. Lett. 8:857–874. doi:10.1111/j.1461-0248.2005.00782.x

Tuck, S. L., C. Winqvist, F. Mota, J. Ahnström, L. A. Turnbull, and J. Bengtsson. 2014. Land-use intensity and the effects of organic farming on biodiversity: A hierarchical meta-analysis. J. Appl. Ecol. 51:746–755. doi:10.1111/1365-2664.12219

Walton, N. J. and R. Isaacs. 2011. Influence of native flowering plant strips on natural enemies and herbivores in adjacent blueberry fields. Environ. Entomol. 40:697–705. doi:10.1603/EN10288

Wolda, H. 1988. Insect seasonality: Why? Annu. Rev. Ecol. Syst. 19:1–18. doi:10.1146/annurev.es.19.110188.000245

Wyss, E., U. Niggli, and W. Nentwig. 1995. The impact of spiders on aphid populations in a strip-managed apple orchard. J. Appl. Entomol. 119:473–478. doi:10.1111/j.1439-0418.1995.tb01320.x

有機與慣行農法對印度棗果園瓢蟲多樣性、優勢度與時間穩定性的影響

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摘要

郝秀花、董耀仁、許北辰、李奇峯、石憲宗。2026。有機與慣行農法對印度棗果園瓢蟲多樣性、優勢度與時間穩定性的影響。台灣農業研究 75(2):217–230。

增進我們對瓢蟲生物多樣性如何應對有機與慣行農法的瞭解，對於熱帶果樹系統的永續性害蟲管理至關重要。本研究旨在比較不同耕作方式下瓢蟲群聚 (assemblages) 之組成與年際變動特性，以評估熱帶果園天敵群聚在有機 (organically cultivated; OC) 與慣行 (conventionally cultivated; CC) 管理下之長期差異。2020−2024 年於高雄燕巢印度棗園進行調查，OC 園共記錄 20 種瓢蟲，顯著高於 CC 園之 11 種。兩類型園區的優勢種皆為臺南方瓢蟲 (*Sasajiscymnus tainanensis*)，而變斑隱勢瓢蟲 (*Cryptogonus orbiculus*) 與六條瓢蟲 (*Cheilomenes sexmaculata*) 在 OC 出現較高豐度，食菌性的黃瓢蟲 (*Illeis koebelei*) 則僅見於 OC 園。每次調查之平均物種豐富度與總豐度在 OC 園皆顯著高於 CC 園 ($P < 0.05$)。OC 園之 Shannon 多樣性 (H') 亦高於 CC 園，而 Berger-Parker 優勢度指數在兩類型間無顯著差異。年總豐度在五年間皆以 OC 園較高，僅 2021 年兩者差異達顯著水準。年際變動方面，OC 園之群聚變異係數 (coefficient of variation; CV = 0.753) 高於 CC 園 (CV = 0.424)，顯示其瓢蟲群聚具有較大的年際波動，反映在低藥劑干擾下族群對獵物資源變化的自然回應。本研究提供熱帶果樹系統中不同耕作管理對瓢蟲群聚多年期差異之實證資料，可作為生態監測及害蟲管理策略規劃之基礎。

關鍵詞：瓢蟲科、生物防治、捕食者群聚、農業集約化、生態系服務。

投稿日期：2025 年 8 月 22 日；接受日期：2026 年 1 月 28 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):231–247 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0005

Research Article

Predatory Lady Beetle Assemblages under Organic and Conventional Farming in Eastern Taiwan's Indigenous Agriculture

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Abstract

Dong, Y. J., P. C. Hsu, C. F. Lee, M. J. Tseng, and H. T. Shih. 2026. Predatory lady beetle assemblages under organic and conventional farming in eastern Taiwan's indigenous agriculture. J. Taiwan Agric. Res. 75(2):231–247.

This study investigated the diversity and temporal stability of lady beetle communities under different farming practices in indigenous agricultural systems of Hualien, eastern Taiwan. From 2022 to 2024, surveys were conducted in organically and conventionally managed rice paddies and pomelo orchards using yellow sticky traps. A total of 29 species were recorded in rice fields and 31 in orchards. Organic management, particularly in perennial pomelo orchards, significantly enhanced lady beetle abundance, species richness, and population stability. In orchards, *Scymnus yangi* Yu et Pang, 1993, and *Sasajiscymnus tainanensis* (Ohta, 1929) were the most dominant species under organic management, while in rice fields, *Micraspis discolor* (Fabricius, 1798) showed higher abundance and stability in organic plots. These results suggest that organic practices can promote beneficial insect populations in indigenous farmlands, especially in orchard systems. The findings provide baseline data for the development of biological control strategies and may inform eco-label certification schemes that incorporate native predatory lady beetles as bioindicators in sustainable agriculture.

Key words: Indigenous farmlands, Organic farming, Conventional farming, Predatory lady beetles, Biodiversity, Stability.

INTRODUCTION

In eastern Taiwan, the relatively slow pace of industrial development has helped preserve low levels of environmental pollution, resulting in superior air quality, clean irrigation water, and healthy soil conditions. These favorable environmental factors provide a solid foundation for promoting environmentally friendly agricultural practices. A diverse population, rich cultural heritage, and picturesque rural landscapes also characterize the region. With both natural and cultural advantages, indigenous areas in eastern Taiwan have strong potential to develop organic and eco-friendly farming systems (Lin *et al.* 2024).

However, in practice, indigenous farmers often refrain from using chemical pesticides and fertilizers in accordance with organic farming principles, which increases their vulnerability to pest infestations. This frequently results in reduced yields and product quality, diminishing

Received: August 15, 2025; Accepted: January 28, 2026.
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farmers' willingness to maintain organic cultivation. Moreover, many tribal farmers lack sufficient technical knowledge of biological and physical pest control, and early warning or rapid response systems for pest outbreaks remain underdeveloped. These limitations have contributed to inconsistent pest management outcomes and represent a major barrier to advancing environmentally friendly agricultural practices in indigenous communities (Yen & Chen 2022).

In Taiwan's indigenous agricultural landscapes, the surrounding habitats are typically less intensively managed and more heterogeneous due to long-standing stewardship and customary land use practices. Such low-disturbance, high-heterogeneity settings support higher biodiversity than intensively farmed regions, with syntheses showing that biodiversity levels on indigenous-managed lands are comparable to- or exceed- those of protected areas (Garnett *et al.* 2018). Biodiversity responses to management are also strongly mediated by landscape heterogeneity, where complex, low-disturbance mosaics favor greater natural-enemy diversity and pest regulation (Batáry *et al.* 2011). In perennial orchard systems, organic management, embedded within heterogeneous landscapes, further enhances biological control compared to conventional systems (Porcel *et al.* 2018).

These relationships are also evident in Taiwan's indigenous farmlands. In the Rukai community of Wutai Village, for instance, traditional practices such as multi-cropping, crop rotation, and periodic fallowing are applied across various altitudes, representing long-term adaptations to mountainous environments. Such strategies maintain soil fertility and enhance biodiversity within indigenous systems (Yen & Chen 2014; Ba *et al.* 2023). The coexistence of low-disturbance habitats and traditional farming methods thus provides a unique setting for the persistence of natural enemies- key components of sustainable pest management.

Among these natural enemies, lady beetles (Family: Coccinellidae) are recognized as one of the most functionally important predator groups (Obrycki & Kring 1998; Symondson *et al.* 2002; Koch 2003). They are polyphagous predators that feed on a broad range of agricultural pests, and their abundance and diversity often reflect the quality of habitat management. As mobile generalist predators, lady beetles play a central role in sustaining natural pest regulation in diversified or low-disturbance farming systems, making them valuable indicators of the effectiveness of biodiversity-based pest control. The family Coccinellidae comprises approximately 6,000 species worldwide, grouped into three major functional categories- predatory, herbivorous, and mycophagous species (Seago *et al.* 2011; Patil & Gaikwad 2019). Among these, predatory species constitute the majority and are known for preying effectively on mites, aphids, thrips, scale insects, psyllids, planthoppers, leafhoppers, and the eggs or early instars of lepidopteran pests. Owing to this role, predatory lady beetles are integral to biological control strategies and remain key agents in integrated pest management (IPM) programs (Symondson *et al.* 2002; Giorgi *et al.* 2009; Megha *et al.* 2015; Kundoo & Khan 2017; Shanker *et al.* 2018; Pervez *et al.* 2020).

According to the Catalogue of Life in Taiwan, a total of 241 species of lady beetles, belonging to 63 genera, have been recorded in Taiwan (TaiCOL 2025). In a recent study of agricultural ecosystems, Dong *et al.* (2023) documented 68 species across 11 agricultural sites, representing 8 subfamilies, 11 tribes, and 32 genera- indicating considerable lady beetle diversity within farmlands.

Despite this diversity, research on lady beetle assemblages within Taiwan's indigenous agricultural systems remains scarce. To address this gap, the present study was conducted from 2022 to 2024 in two indigenous communities of Hualien County, eastern Taiwan. Lady beetle populations were systematically monitored under both organic and conventional management in the rice paddies of the Ceroh Tribe (Yuli Township) and the pomelo orchards of the Olalip Tribe (Ruisui Township). This study aims to establish baseline data on the species composition and

abundance of lady beetles in tribal farmlands, providing essential references for promoting chemical-free agriculture and biological pest control among indigenous farmers, while supporting ecological intensification and the development of biodiversity-based eco-labeling strategies for indigenous agriculture.

MATERIALS AND METHODS

Study sites and plot design

This study was conducted in two indigenous communities in Hualien County, eastern Taiwan, to investigate lady beetle populations. The survey sites included rice paddies in the Ceroh community of Chunri Village, Yuli Township, and pomelo orchards in the Olalip community of Hegang Village, Ruisui Township. In the Ceroh community, five organically managed rice fields were selected as sampling plots, located at the following coordinates: 23.447174°N, 121.393555°E; 23.446947°N, 121.393872°E; 23.449429°N, 121.397979°E; 23.455473°N, 121.402310°E; and 23.456656°N, 121.381532°E. In addition, five conventionally managed rice fields were surveyed at: 23.450590°N, 121.396706°E; 23.450955°N, 121.395676°E; 23.450296°N, 121.400425°E; 23.449523°N, 121.400985°E; and 23.458473°N, 121.382067°E. In the Olalip community, four organically managed pomelo orchards were selected at: 23.510089°N, 121.390830°E; 23.511996°N, 121.395446°E; 23.548905°N, 121.392892°E; and 23.548541°N, 121.396226°E. Another four conventionally managed pomelo orchards were surveyed at: 23.504307°N, 121.393696°E; 23.508642°N, 121.392785°E; 23.510239°N, 121.398667°E; and 23.514154°N, 121.394150°E.

All field sites had well-defined management histories prior to the initiation of this study. Among the five organic rice fields, only one held formal organic certification, while the remaining four had been managed under organic or eco-friendly practices without certification. All pomelo orchards were certified organic and had been managed under organic or eco-friendly practices for at least seven years. In contrast, the conventionally managed rice and pomelo orchards had been maintained under pesticide-based practices for more than seven years.

To clarify the spatial setting and experimental design, all selected plots were located within the same indigenous communities, thereby ensuring that organic and conventional fields were compared under the socio-ecological context of each community. In the Ceroh community, organically and conventionally managed rice fields were both surveyed, while in the Olalip community, paired comparisons were made between organically and conventionally managed pomelo orchards. Within each community, the individual fields or orchards were treated as independent replicates, and the organic-versus-conventional contrast was assessed at the community level rather than across distant sites.

In this study, "organically managed fields" refer to agricultural areas managed in accordance with the definition provided in Article 3, Chapter 1 of the Organic Agriculture Promotion Act of Taiwan: "Organic agriculture refers to agricultural practices- including crop cultivation, forestry, aquaculture, and livestock farming- that follow the principles of ecological balance and nutrient cycling, and in which synthetic chemical fertilizers, chemical pesticides, genetically modified organisms (GMOs), and their derivatives are not used during the production process." In practice, organically managed rice paddies and pomelo orchards in the surveyed regions were cultivated strictly in accordance with these regulations. Local farmers emphasized ecological balance and sustainability, refrained from using synthetic fertilizers or pesticides, and instead regularly used environmentally friendly plant protection materials and organic fertilizers throughout the cropping season to prevent or suppress pest and disease outbreaks.

By contrast, "conventionally managed fields" refer to areas where farmers use chemical fertilizers and pesticides for crop cultivation and pest control. In the surveyed rice paddies and pomelo orchards, conventional management followed fertilizer regimes recommended for rice and pomelo production, combined with

farmers' routine practices. Chemical fertilizers were applied during crop growth, and pesticides were sprayed at scheduled intervals. When pest infestations became severe, the frequency of pesticide applications was increased to suppress pest and disease populations. These management regimes were representative of typical practices in the indigenous farming communities of eastern Taiwan.

Pesticide usage in rice paddies and pomelo orchards

Pesticide regimes for each management type were documented through interviews with farmers and field records. In conventionally managed rice paddies, pesticides were applied only once per crop cycle, typically around the fourth week after transplanting during the tillering stage. The single application included a mixture of insecticides and fungicides such as pyrethroids (cyhalothrin), organochlorines (endosulfan), and organophosphate fungicides (edifenphos and tricyclazole). No additional sprays were applied before or after the peak activity periods of lady beetles. In contrast, organically managed rice fields received no chemical pesticide applications throughout the study period.

In pomelo orchards, conventional management followed a substantially more intensive pesticide regime. From December to the following August, growers applied pesticides 1–2 times per month, depending on pest or disease pressure, with each spray involving 1–3 active ingredients. Common insecticides included organophosphates (malathion, chlorpyrifos), avermectins (abamectin), carbamates (carbaryl), pyrethroids (cyhalothrin, deltamethrin), neonicotinoids (imidacloprid, acetamiprid), and insect growth regulators (pyriproxyfen). Mite control relied on acaricides such as fenpyroximate, while disease management involved repeated applications of fungicides, including mancozeb, zineb, hymexazol, triflumizole, chlorothalonil, and difenoconazole. Organic pomelo orchards, by contrast, applied only environmentally friendly plant-derived materials- primarily narrow-range oil, neem oil, and potassium phosphite mixtures- at similar seasonal intervals.

These system-level differences in pesticide intensity provide essential contextual information for interpreting lady beetle responses under organic and conventional management. Conventional rice fields experienced only minimal chemical disturbance, whereas conventional pomelo orchards were exposed to pesticides for most of the year. This contrast in cumulative spray intensity helps explain why organic management produced much stronger community-level differences in pomelo orchards than in rice systems.

Survey methods

Lady beetle monitoring was conducted from 2022 to 2024 using yellow sticky papers, with surveys conducted at two-month intervals. The deployment and sampling protocols for yellow sticky traps in both rice paddies and pomelo orchards followed the standardized methods described by Dong *et al.* (2023). In the rice fields, five organically managed plots and five conventionally managed plots were selected for study. During each sampling event, four yellow sticky traps were installed in each plot, totaling 40 traps per sampling (5 plots × 2 management types × 4 traps). Similarly, in the pomelo orchards, four organic and four conventional plots were surveyed, with four traps installed per plot, resulting in a total of 32 traps per sampling event (4 plots × 2 management types × 4 traps). Trap placement within each plot followed a stratified random design to minimize microhabitat bias while maintaining consistency across sampling periods.

In rice paddies, traps were installed slightly above the canopy at each sampling event to account for plant growth over time, ensuring consistent exposure to flying insects across different crop stages. In pomelo orchards, traps were fixed at approximately chest height (~1.5 m above ground). Yellow sticky papers were replaced regularly, and all specimens adhering to the yellow sticky papers were collected

and brought back to the laboratory for sorting and identification. Captured lady beetles were identified to the species level and recorded numbers for subsequent analysis. The mean abundance of each lady beetle species was calculated as the average number of individuals per trap per sampling event across each site × year combination (i.e., total individuals/number of traps/number of sampling months). This standardization allowed comparisons across plots and years with consistent trapping effort.

Statistical analysis

This study conducted statistical analyses to evaluate the community composition, diversity, and temporal stability of lady beetle populations under different farming practices. The following indices and methods were used:

Shannon diversity index (*H'*): This index measures species diversity and evenness within each sampling plot. Higher H' values indicate greater species richness and a more even distribution of individuals among species (Magurran 2004).

Berger-Parker dominance index (*d*): This index quantifies the proportion of individuals belonging to the most abundant species. Lower values of d suggest a more balanced community structure with less dominance by a single species (Berger & Parker 1970).

Coefficient of variation (CV): Used to assess interannual variability and temporal stability of lady beetle populations. It is calculated as the standard deviation divided by the mean (CV = *SD*/Mean), with lower CV values indicating greater population stability over time (Tilman 1999).

To compare differences between organic and conventional fields in terms of diversity indexes (H' and d) and total lady beetle abundance, non-parametric Mann-Whitney U tests were performed. The significance level was set at $P < 0.05$. All statistical analyses were conducted using R software (version 4.3.0; R Core Team, 2023). Significant differences between organic and conventional fields were indicated in tables using asterisks ($^{*}P < 0.05$, $^{**}P < 0.01$). Supplementary bar charts were generated to visualize differences in the abundance and diversity of dominant species.

RESULTS

Paddy fields (Table 1). A total of 29 lady beetle species were recorded in paddy fields during 2022–2024, forming a predator-dominated assemblage. The community was mainly composed of predatory taxa, among which *Micraspis discolor* (Fabricius, 1798) was the most abundant (14.2 ± 1.49 individuals trap^{-1} event^{-1}), representing about 55% of all captures. *Propylea japonica* (Thunberg, 1781) (2.68 ± 0.38; 10%) and *Cheilomenes sexmaculata* (Fabricius, 1781) (2.07 ± 0.50; 8%) were the next most common species, followed by *Scymnus hoffmanni* Weise, 1879 (1.84 ± 0.43; 7%) and *Lemnia biplagiata* (Swartz, 1808) (0.32 ± 0.07; 1%). Together, these five predatory species accounted for roughly 81% of the total individuals, indicating a highly uneven dominance structure. In addition, one fungivorous species (*Illeis koebelei* Timberlake, 1943) and three herbivorous species- *Hensepilachna vigintioctopunctata* (Fabricius, 1775), *Epilachna maxima* (Weise, 1898), and *E. hendecaspilota* (Mader, 1927)- were recorded but occurred at very low abundances (< 0.1 individuals trap^{-1} event^{-1}). Overall, the paddy-field lady beetle community was strongly dominated by predatory species, with only a minor presence of herbivorous and fungivorous taxa.

Pomelo orchards (Table 2). A total of 31 lady beetle species were recorded in pomelo orchards during 2022–2024, forming a predator-dominated assemblage. The community was mainly composed of predatory taxa, with the five most abundant species being *Sc. yangi* Yu et Pang, 1993, *Sasajiscymnus tainanensis* (Ohta, 1929), *Sticholotis formosana* Weise, 1923, *Cryptogonus orbiculus* (Gyllenhal, 1808), and *Axinoscymnus nigripennis* Kamiya, 1965, which together accounted for over 70% of total captures. Among these, *Sc. yangi* (1.18 ± 0.32 individuals trap^{-1} event^{-1}) and *Sa. tainanensis*

Table 1. Lady beetle species and their mean abundance in paddy fields from 2022 to 2024 (ranked by abundance).

Scientific name	Mean abundance (mean ± *SE*)[z]
Micraspis discolor (Fabricius, 1798)[y]	14.2 ± 1.49
Propylea japonica (Thunberg, 1781)[y]	2.68 ± 0.38
Cheilomenes sexmaculata (Fabricius, 1781)	2.07 ± 0.50
Scymnus hoffmanni Weise, 1879[y]	1.84 ± 0.43
Lemnia biplagiata (Swartz, 1808)	0.32 ± 0.07
Harmonia octomaculata (Fabricius, 1781)	0.21 ± 0.04
Cryptogonus orbiculus (Gyllenhal, 1808)[y]	0.14 ± 0.04
Sasajiscymnus tainanensis (Ohta, 1929)[y]	0.13 ± 0.03
Sc. yangi Yu et Pang, 1993[y]	0.06 ± 0.02
Hensepilachna vigintioctopunctata (Fabricius, 1775)[y]	0.06 ± 0.02
Platynaspidius maculosus (Weise, 1910)[y]	0.05 ± 0.02
Coccinella transversalis Fabricius, 1781	0.04 ± 0.02
L. saucia (Mulsant, 1850)	0.03 ± 0.02
Sticholotis formosana Weise, 1923[y]	0.02 ± 0.01
Stethorus aptus Kapur, 1948[y]	0.02 ± 0.01
Sc. posticalis Sicard, 1912[y]	0.02 ± 0.02
Jauravia limbata Motschulsky, 1858[y]	0.02 ± 0.02
Sc. quadrillum Motschulsky, 1858	0.02 ± 0.01
Nephus tagiapatus (Kamiya, 1961)	0.01 ± 0.01
Olla v-nigrum (Mulsant, 1866)	0.01 ± 0.01
Coelophora inaequalis (Fabricius, 1775)[y]	0.01 ± 0.01
Cr. montrouzieri Mulsant, 1853	0.01 ± 0.01
Epilachna maxima (Weise, 1898)	0.01 ± 0.01
Amida tricolor formosana Kurisaki, 1920[y]	0.01 ± 0.01
Illeis koebelei Timberlake, 1943[y]	0.01 ± 0.01
Oenopia sauzeti Mulsant, 1866	0.01 ± 0.01
Oe. formosana (Miyatake, 1965)[y]	0.01 ± 0.01
E. hendecaspilota (Mader, 1927)	0.01 ± 0.01
Sa. fuscus (Yang, 1971)	0.01 ± 0.01

[z] This table presents the average number of lady beetles (Coccinellidae) captured in organic and conventional paddy fields from 2022 to 2024. Mean abundance = average number per trap per sampling event (see Methods for details).
[y] Indicates species occurring in both paddy fields and pomelo orchards.

(1.02 ± 0.18) were the two predominant species. In addition to the predatory taxa, one fungivorous species (*I. koebelei*) and two herbivorous species- *H. vigintioctopunctata* and *E. chinensis* (Weise, 1912)- were also recorded, though all occurred only occasionally at very low abundance (< 0.05 individuals trap^{-1} event^{-1}). The pomelo-orchard community exhibited a more pronounced predator-dominated pattern, with only occasional records of herbivorous and fungivorous species.

Across the two agricultural environments, 17 lady beetle species were shared between rice paddies and pomelo orchards: *M. discolor*, *P. japonica*, *Ch. sexmaculata*, *Sc. hoffmanni*, *Sc. yangi*, *Sc. posticalis* Sicard, 1912, *Sti. formosana*, *Stechorus aptus* Kapur, 1948, *Sa. tainanensis*, *Cr. orbiculus*, *I. koebelei*, *Amida tricolor formosana* Kurisaki, 1920, *Jauravia limbata* Motschulsky, 1858, *Platynaspidius maculosus* (Weise, 1910), *Coelophora inaequalis* (Fabricius,

Table 2. Lady beetle species and their mean abundance in pomelo orchards from 2022 to 2024 (ranked by abundance).

Scientific name	Mean abundance (mean ± *SE*)[z]
Scymnus yangi Yu et Pang, 1993[y]	1.18 ± 0.32
Sasajiscymnus tainanensis (Ohta, 1929)[y]	1.02 ± 0.18
Sticholotis formosana Weise, 1923[y]	0.51 ± 0.13
Cryptogonus orbiculus (Gyllenhal, 1808)[y]	0.33 ± 0.08
Axinoscymnus nigripennis Kamiya, 1965	0.22 ± 0.09
Sa. orbiculatus (Yang, 1971)	0.21 ± 0.09
Sc. centralis Kamiya, 1965	0.19 ± 0.07
Stethorus aptus Kapur, 1948[y]	0.19 ± 0.08
Cheilomenes sexmaculata (Fabricius, 1781)[y]	0.15 ± 0.05
Sa. parenthesis (Weise, 1923)	0.10 ± 0.03
Rodolia okinawensis Miyatake, 1959	0.08 ± 0.04
Propylea japonica (Thunberg, 1781)[y]	0.07 ± 0.03
Illeis koebelei Timberlake, 1943[y]	0.06 ± 0.02
Hensepilachna vigintioctopunctata (Fabricius, 1775)[y]	0.05 ± 0.02
Sc. hoffmanni Weise, 1879[y]	0.04 ± 0.03
St. loi Sasaji, 1968	0.03 ± 0.03
Micraspis discolor (Fabricius, 1798)[y]	0.03 ± 0.02
R. cardinalis (Mulsant, 1850)	0.03 ± 0.01
Platynaspidius quinquepunctatus Miyatake, 1961	0.02 ± 0.01
Amida tricolor formosana Kurisaki, 1920[y]	0.02 ± 0.01
Jauravia limbata Motschulsky, 1858[y]	0.02 ± 0.02
Nesolotis shirozui Sasaji, 1967	0.01 ± 0.01
Coelophora inaequalis (Fabricius, 1775)[y]	0.01 ± 0.01
Pl. maculosus (Weise, 1910)[y]	0.01 ± 0.01
Sc. posticalis Sicard, 1912[y]	0.01 ± 0.01
Oenopia formosana (Miyatake, 1965)[y]	0.01 ± 0.01
Sa. quinquepunctatus (Weise, 1923)	0.01 ± 0.01
St. hirashimai Sasaji, 1967	0.01 ± 0.01
Aspidimerus esakii Sasaji, 1968	0.01 ± 0.01
Cr. kurosawai Sasaji, 1968	0.01 ± 0.01
Epilachna chinensis (Weise, 1912)	0.01 ± 0.01

[z] Mean abundance = average number per trap per sampling event (see Methods for details).
[y] Indicates species occurring in both paddy fields and pomelo orchards.

1775), *Oenopia formosana* (Miyatake, 1965), and *H. vigintioctopunctata*. In contrast, several species were recorded exclusively in one habitat type, such as *A. nigripennis* and *Nesolotis shirozui* Sasaji, 1967 in pomelo orchards, and *L. biplagiata* and *Harmonia octomaculata* (Fabricius, 1781) in rice fields. The occurrence of both shared and habitat-specific species indicates distinct species assemblages between annual (paddy) and perennial (orchard) systems.

Dominant species in rice fields (Table 3). Organic management in rice fields significantly altered the composition of the lady beetle community. *M. discolor* showed a 2.2-fold

higher abundance in organic (OC) rice fields compared with conventional (CC) fields (19.50 ± 2.54 vs. 8.90 ± 1.36 individuals trap^{-1} event^{-1}), corresponding to a 119% increase relative to CC fields. This single species accounted for approximately 75% of the total lady beetle abundance in organic rice systems, indicating a strongly dominance-driven community structure. In contrast, *Ch. sexmaculata* showed slightly higher abundance in conventional fields (2.88 ± 0.96 vs. 1.27 ± 0.23), though this difference was not statistically significant (P = 0.213).

Dominant species in pomelo (Table 4). In pomelo orchards, all five major predatory species- *Sc. yangi*, *Sa. tainanensis*, *Sti. formosana*, *Cr. orbiculus*, and *A. nigripennis*- were significantly more abundant in organically managed (OC) than in conventionally managed (CC) orchards (5 of 5 species; 100% significant; all P < 0.001). Among these, *Sc. yangi* and *Sa. tainanensis* were the most dominant, with mean abundances of 2.32 ± 0.62 and 1.76 ± 0.32 individuals trap^{-1} event^{-1} in OC orchards, respectively, compared with < 0.3 in CC orchards. The co-dominance of these two species and the consistent presence of other predators indicate a more even and stable assemblage structure under organic management. In contrast, CC orchards exhibited a highly uneven community composition, characterized by the near absence of these dominant predators.

Event-level (In this analysis, each "event" corresponds to one standardized sampling occasion (every two months), representing temporal variation across crop stages and across years.) Comparisons between organic (OC) and conventional (CC) management (Table 5) revealed clear differences in community stability and diversity. Across all sites, OC fields exhibited higher Shannon diversity (H': = 0.763 ± 0.042 vs. 0.490 ± 0.042; U = 16,974.5, P <

Table 3. Analysis of dominant lady beetle species under organic (OC) and conventional (CC) management in rice fields.

Scientific name	Mean abundance (mean ± SE) OC	CC	U statistic	P-value[z]
Micraspis discolor (Fabricius, 1798)	19.50 ± 2.54**	8.90 ± 1.36	5,471.5	< 0.0001
Propylea japonica (Thunberg, 1781)	2.48 ± 0.34	2.89 ± 0.68	4,257.0	0.5431
Cheilomenes sexmaculata (Fabricius, 1781)	1.27 ± 0.23	2.88 ± 0.96	3,649.5	0.2134
Scymnus hoffmanni Weise, 1879	1.72 ± 0.64	1.96 ± 0.56	3,775.0	0.3554
Lemnia biplagiata (Swartz, 1808)	0.27 ± 0.09	0.37 ± 0.10	3,863.5	0.4118

[z] Mann-Whitney U test was used to compare the mean number of lady beetles between organic and conventional treatments. Mean abundance = average number per trap per sampling event (see Methods for details).
*Significance codes: *P < 0.05, **P < 0.01. Values with different superscript letters differ significantly between management types.

Table 4. Analysis of dominant lady beetle species under organic (OC) and conventional (CC) management in pomelo orchards.

Scientific name	Mean abundance (mean ± SE) OC	CC	U statistic	P-value[z]
Scymnus yangi Yu et Pang, 1993	2.32 ± 0.62**	0.04 ± 0.02	3,628.50	< 0.0001
Sasajiscymnus tainanensis (Ohta, 1929)	1.76 ± 0.32**	0.28 ± 0.12	3,755.50	< 0.0001
Sticholotis formosana Weise, 1923	1.00 ± 0.26**	0.01 ± 0.01	3,858.50	< 0.0001
Cryptogonus orbiculus (Gyllenhal, 1808)	0.64 ± 0.14**	0.03 ± 0.02	3,396.00	< 0.0001
Axinoscymnus nigripennis Kamiya, 1965	0.44 ± 0.17**	0.00 ± 0.00	3,024.00	0.0003

[z] Mann-Whitney U test was used to compare the mean number of lady beetles between organic and conventional treatments. Mean abundance = average number per trap per sampling event (see Methods for details).
*Significance codes: *P < 0.05, **P < 0.01. Values with different superscripts differ significantly across management types.

Table 5. Comparison of coefficients of variation (CV) and Mann-Whitney *U* test results for three stability indicators of lady beetle populations between organic (OC) and conventional (CC) management.

	Mean Shannon (*SE*)				CV (*SE*)	
Stability indicator/sampling category	OC	CC	*U* statistic	*P*-value[z] (Cohen's *d*)	OC	CC
Shannon diversity index, (H')						
Overall	0.763** (0.042)	0.490 (0.042)	16,974.5	< 0.0001 (0.51)	0.697 (0.054)	1.089 (0.111)
Rice fields	0.665* (0.049)	0.814 (0.051)	3,358.5	0.0475 (-0.32)	0.704 (0.074)	0.591 (0.057)
Pomelo orchards	0.886** (0.069)	0.085 (0.028)	4,455.5	< 0.0001 (1.79)	0.657 (0.075)	2.799 (0.952)
Total lady beetle abundance (*N*)						
Overall	18.250** (1.850)	10.300 (1.430)	17,850.5	< 0.0001 (0.38)	1.287 (0.148)	1.770 (0.265)
Rice fields	26.010* (2.930)	17.930 (2.280)	4,920.0	0.0126 (0.40)	1.069 (0.144)	1.204 (0.177)
Pomelo orchards	8.560** (1.240)	0.750 (0.180)	4,691.5	< 0.0001 (1.14)	1.232 (0.206)	2.084 (0.541)
Berger-Parker dominance index (*d*)						
Overall	12.930** (1.550)	6.680 (0.980)	17,701.5	< 0.0001 (0.29)	1.526 (0.202)	1.869 (0.293)
Rice fields	19.860* (2.520)	11.540 (1.590)	5,131.0	0.0020 (0.44)	1.204 (0.177)	1.304 (0.204)
Pomelo orchards	4.260** (0.630)	0.600 (0.150)	4,579.0	< 0.0001 (0.94)	1.246 (0.210)	2.115 (0.556)

[z] Mann-Whitney *U* test was used to compare the mean number of lady beetles between organic and conventional treatments. Effect sizes (Cohen's *d*) were calculated as the difference between means divided by the pooled standard deviation to complement *P*-values (Cohen 1988). Values of d ≈ 0.2, 0.5, and 0.8 correspond to small, medium, and large effects, respectively. Positive values of Cohen's *d* indicate higher means in organic (OC) fields, whereas negative values indicate higher means in conventional (CC) fields.
*Significance codes: $^{*}P < 0.05$, $^{**}P < 0.01$. Values with different superscripts differ significantly across management types.

0.0001, Cohen's $d = 0.51$ – medium effect) and greater total abundance (18.25 ± 1.85 vs. 10.30 ± 1.43; $U = 17{,}850.5$, $P < 0.0001$, $d = 0.38$ – small-to-medium effect). In rice fields, diversity was slightly higher in CC plots (H': = 0.814 ± 0.051 vs. 0.665 ± 0.049; $U = 3{,}358.5$, $P = 0.0475$, $d = -0.32$ – small negative effect), suggesting limited sensitivity of the rice assemblage to organic management. In contrast, pomelo orchards showed a pronounced positive response, with OC fields having markedly greater Shannon diversity (H': = 0.886 ± 0.069 vs. 0.085 ± 0.028; $U = 4{,}455.5$, $P < 0.0001$, $d = 1.79$ – large effect) and abundance (8.56 ± 1.24 vs. 0.75 ± 0.18; $d = 1.14$). To improve interpretability, significant contrasts between organic and conventional management shown in Tables 3–5 were also visualized in supplementary bar charts (Supplementary Figs. 1 and 2).

Coefficients of variation (CV) corroborated these patterns: OC fields, particularly in pomelo orchards, exhibited lower temporal variability (Overall: 0.697 vs. 1.089; Pomelo: 0.657 vs. 2.799), indicating greater population stability under organic management. Overall, both statistical significance and effect-size strength confirm that organic management enhances coccinellid diversity and stability, especially in perennial orchard systems.

DISCUSSION

Species composition and cross-system patterns

This study revealed a diverse assemblage of lady beetles across both rice and pomelo systems within the indigenous agricultural landscapes of Hualien. Seventeen species were shared between the two systems, including generalist predators such as *Ch. sexmaculata* (Fabricius, 1781) and *M. discolor* (Fabricius, 1798), reflecting broad ecological tolerance and cross-habitat persistence among predatory taxa. Consistent with the data in Tables 1 and 2, these traditional farmlands supported a predominantly predatory coccinellid community, underscoring the ecological integrity maintained under low-intensity, community-based management.

Comparative evidence from tropical Asia shows partially overlapping species composition with that of Taiwan's indigenous agroecosystems. In India's organic vegetable farms, *Coccinella transversalis* Fabricius, 1781, *Ch. sexmaculata*, *H. octomaculata* (Fabricius, 1781), and *M. discolor* dominate under low-input management (Rajan *et al.* 2019), suggesting that tropical Asian systems share a core guild of generalist predators adapted to reduced-chemical environments. In contrast, temperate and Neotropical regions exhibit distinct predator assemblages- *Coccinella septempunctata* Linnaeus, 1758, *Tytthaspis sedecimpunctata* Linnaeus, 1758, and *Scymnus* spp. in Ukraine (Grabovska *et al.* 2021) and *Rodolia cardinalis* (Mulsant, 1850) in Brazilian citrus orchards (de Paula Malheiros *et al.* 2025). The exclusive occurrence of *R. cardinalis* in our pomelo orchards reinforces its citrus specialization. Together, these regional contrasts illustrate how both shared and habitat-specific predators shape the broader ecological mosaic of indigenous farmlands.

Ecological differentiation and resilience

The coexistence of shared and habitat-specific lady beetles reflects distinct ecological filters between annual and perennial systems. Shared taxa such as *Ch. sexmaculata* and *M. discolor* demonstrate cross-habitat persistence, while orchard- or paddy-restricted species likely represent localized adaptation or prey specialization. This differentiation aligns with studies showing that diversified or perennial habitats promote predator persistence and ecological stability (Symondson *et al.* 2002; Crowder *et al.* 2010; Tscharntke *et al.* 2012; Porcel *et al.* 2018). Collectively, generalists ensure continuity across habitats, whereas specialists sustain localized stability- together enhancing the resilience of indigenous farming landscapes.

Global parallels and habitat complexity

Similar biodiversity patterns have been widely reported in organic systems worldwide, where reduced pesticide use and diversified vegetation promote higher predator abundance and community stability (Porcel *et al.* 2018; Grabovska *et al.* 2021; de Paula Malheiros *et al.* 2025). Studies from Asia and other subtropical regions, which enhance natural pest regulation (Chouangthavy *et al.* 2021; Chouangthavy & Fourcade 2024). These parallels suggest that the indigenous farmlands of eastern Taiwan functionally resemble mature organic systems in other tropical regions, despite their smaller scale and community-based management.

This pattern accords with the "habitat complexity-stability" relationship in landscape ecology. Perennial orchards- with their greater structural complexity and year-round continuity- provide more favorable habitats for natural enemies than short-cycle rice fields (Tscharntke *et al.* 2012; Rada *et al.* 2023). Structural and temporal heterogeneity within these indigenous farmlands thus enhances predator stability and reinforces ecological resilience under low-input management.

Biogeographic distinctiveness and endemic diversity

The limited overlap with temperate or Neotropical systems underscores the biogeographic uniqueness of Taiwan's indigenous

farmlands. Situated at the subtropical–tropical transition, these landscapes host both Oriental and island-endemic predator lineages such as *Sc. yangi* Yu et Pang, 1993, *Sa. tainanensis* (Ohta, 1929), and *A. nigripennis* Kamiya, 1965- taxa seldom recorded elsewhere. Their coexistence with cosmopolitan species, such as *Ch. sexmaculata* and *M. discolor*, forms a mosaic community that integrates local adaptation and broad ecological tolerance. This pattern reflects the heterogeneous, low-intensity management characteristic of indigenous farming (Yen & Chen 2014; Ba *et al.* 2023), positioning these systems as valuable references for understanding predator persistence and trophic stability in traditional agroecosystems.

Functional mechanisms and stability patterns

The functional mechanisms underlying these assemblages have been elucidated through stable-isotope and diet-based studies in Taiwanese rice systems. Hsu *et al.* (2021) demonstrated that generalist predators shift trophic niches seasonally, consuming more herbivorous prey in conventional fields where pest densities are higher. Landscape complexity further enhances predator abundance and prey availability (Ali *et al.* 2020; Saqib *et al.* 2022). These findings indicate that heterogeneous, low-disturbance landscapes typical of indigenous areas facilitate predator persistence and flexible foraging that track pest pulses, thereby enhancing biodiversity-based pest regulation.

Within this trophic framework, *M. discolor* consistently emerged as a keystone predator maintaining pest suppression under fluctuating prey conditions (Shanker *et al.* 2013; Poorani *et al.* 2023; Chouangthavy & Fourcade 2024). Beyond *M. discolor*, other predatory coccinellids also responded positively to organic management, contributing complementary roles in stability and resilience (Letourneau *et al.* 2011; Porcel *et al.* 2018; Grabovska *et al.* 2021; Rada *et al.* 2023). In perennial pomelo orchards, *Sc. yangi*, *Sa. tainanensis*, and *A. nigripennis* were closely associated with pesticide-free, structurally complex habitats, serving as reliable indicators of reduced-chemical environments. Together, these results demonstrate that resilience in indigenous agroecosystems is achieved through dual stability mechanisms- dominance-driven in annual rice systems and diversity-driven in perennial orchards.

Community stability

Quantitative stability indices (Table 5) revealed that organic management generally enhanced lady beetle stability, particularly in orchard systems. In contrast, rice fields exhibited lower diversity under organic management due to the dominance of *M. discolor*. While such dominance reduces evenness, it ensures strong pest control- a trade-off widely observed in tropical agroecosystems (Shanker *et al.* 2013; Grabovska *et al.* 2021; Rada *et al.* 2023).

Previous studies have shown that predatory lady beetles adjust their diets according to prey availability and crop phenology. Hsu *et al.* (2025) reported that predator foraging behavior in rice fields shifts substantially across growth stages, with broader prey use in organic systems due to higher habitat heterogeneity and alternative prey sources. Such seasonal and habitat-mediated flexibility likely also operates in our indigenous agricultural systems.

Seasonal peaks in herbivore availability during the flowering-maturation stages likely shape the foraging dynamics of predatory lady beetles in both rice and orchard systems. Meanwhile, surrounding semi-natural habitats within indigenous agricultural landscapes provide alternative prey and microhabitats that buffer population fluctuations and promote trophic resilience. These landscape-level features may explain why organic orchard systems showed high temporal stability, whereas organic rice fields were more strongly affected by single-species dominance.

Dominant predator function and trophic stability

Across management regimes, *M. discolor* demonstrated consistent functional stability, preying heavily on rice herbivores throughout

ripening (Hsu *et al.* 2025). This persistent prey specialization underlines its role as a dominant "pest specialist" capable of sustaining top-down control even when community diversity is reduced. Such dominance-based stability mechanisms are well documented in other low-input systems (Chouangthavy *et al.* 2021; Grabovska *et al.* 2021). Collectively, these findings emphasize that community-level trophic flexibility and the persistence of efficient predator species together ensure reliable pest suppression in indigenous and organic rice ecosystems.

In addition, all survey sites were located within indigenous agricultural landscapes of eastern Taiwan, where forest cover, landscape heterogeneity, and traditional low-input farming practices contribute to substantially lower anthropogenic disturbance compared with Taiwan's highly intensified western plains (Chen *et al.* 2021). Such regional contrasts are ecologically meaningful. International syntheses show that biodiversity responses to organic management are strongly context-dependent: organic systems yield larger gains in structurally complex, low-disturbance landscapes than in simplified high-intensity regions (Tuck *et al.* 2014). Likewise, landscape-level disturbance filters strongly influence natural-enemy diversity and trophic stability across agricultural ecosystems (Tscharntke *et al.* 2005). Our findings align with this landscape-context framework. The strong predator stability observed in organic orchards and the dominance-driven patterns in organic rice fields likely reflect interactions between farming practice and the unique low-disturbance setting of indigenous territories.

Ecological intensification and landscape moderation frameworks

The observed dynamics closely correspond to the "ecological intensification" and "landscape moderation" frameworks (Crowder *et al.* 2010; Tscharntke *et al.* 2012). The former proposes that enhanced on-farm biodiversity can substitute for chemical inputs to sustain yields and stability, while the latter predicts that organic benefits are strongest in heterogeneous landscapes and weaker in simplified ones (Bengtsson *et al.* 2005; Batáry *et al.* 2011). Perennial pomelo orchards exemplify ecological intensification through long-term organic management and vegetation complexity, whereas short-cycle rice systems align with the landscape moderation hypothesis, showing weaker diversity responses. Together, these frameworks explain how biodiversity outcomes are jointly shaped by local management and surrounding landscape context.

Indicator species and certification perspectives

Several predatory coccinellids- *Sc. yangi*, *Sa. tainanensis*, *Sti. formosana* Weise, 1923, *Cr. orbiculus* (Gyllenhal, 1808), *A. nigripennis*, and *M. discolor*- show strong affinity to organic systems, suggesting their potential as bioindicators of ecological integrity. Rather than relying on a single species, a multi-species indicator framework would provide a more accurate reflection of functional resilience. Similar models elsewhere show that organic and low-input farms sustain higher predator diversity and more stable community dynamics (Porcel *et al.* 2018; Grabovska *et al.* 2021; Rada *et al.* 2023). Inspired by Finland's "Ladybird Label," a locally adapted certification system could highlight native beetles- *A. nigripennis* for orchards and *M. discolor* for rice- to communicate biodiversity outcomes and strengthen consumer trust. Integrating scientific and cultural symbols would reinforce both ecological branding and the socio-economic sustainability of indigenous organic agriculture.

Future research and management implications

Variability in rice fields indicates that the benefits of organic management are context-dependent, influenced by crop type, local environment, and surrounding landscape configuration (Bengtsson *et al.* 2005; Batáry *et al.* 2011). Future studies should integrate long-

term monitoring, functional trait analyses, and landscape-scale approaches to clarify these interactions. Such integrative research will strengthen evidence-based strategies that guide organic transitions and promote biodiversity-friendly management in Taiwan's indigenous agricultural landscapes.

CONCLUSIONS

This study provides the first multi-year documentation of lady beetle assemblages within indigenous farming systems of eastern Taiwan, recording 29 species in rice paddies and 31 in pomelo orchards from 2022 to 2024. Organic management generally supported higher species richness, abundance, and population stability-particularly in perennial orchard systems. In organically managed pomelo orchards, several dominant species, including *Sc. yangi* Yu et Pang, 1993, and *A. nigripennis* Kamiya, 1965, showed significantly higher or exclusive occurrence, underscoring the ecological benefits of reduced-chemical practices. In rice paddies, *M. discolor* (Fabricius, 1798) emerged as a consistently dominant and stable predator, emphasizing its role as a key biocontrol agent in low-pesticide rice ecosystems.

The integration of diversity and stability indicators demonstrates the value of lady beetles as bioindicators for agroecological assessment. Moreover, these findings provide a robust baseline for developing eco-labeling or certification strategies grounded in native biodiversity, thereby enhancing both the visibility and economic viability of organic produce from indigenous communities.

However, the effects of organic farming were not uniform across crop types. The slightly lower diversity observed in organically managed rice fields suggests that local landscape context, habitat structure, and ecological infrastructure modulate biodiversity outcomes. Future research should therefore integrate landscape-scale, trait-based approaches to elucidate predator–prey dynamics more and support evidence-based policy design for sustainable, biodiversity-friendly agriculture in Taiwan's indigenous regions.

ACKNOWLEDGMENTS

This study was financially supported by the Ministry of Agriculture, Taiwan, under the Scientific Research Projects 114-AgriSci-9.2.3-Bio-01 and 114-AgriSci-4.4.2-Agri-02. The authors sincerely thank the Ministry for its generous support.

REFERENCES

Ali, M. P., M. M. M. Kabir, S. S. Haque, S. Afrin, N. Ahmed, B. Pittendrigh, and X. Qin. 2020. Surrounding landscape influences the abundance of insect predators in rice field. BMC Zool. 5:8. doi:10.1186/s40850-020-00059-1

Ba, Q. X., H. Z. Wang, and M. H. Wang. 2023. Agrobiodiversity, social institutions, and indigenous farming practices: A case study of the Rukai in Wutai, Taiwan. Hum. Ecol. 51:1127–1140. doi:10.1007/s10745-023-00463-4

Batáry, P., A. Báldi, D. Kleijn, and T. Tscharntke. 2011. Landscape-moderated biodiversity effects of agri-environmental management: A meta-analysis. Proc. Biol. Sci. B 278:1894–1902. doi:10.1098/rspb.2010.1923

Bengtsson, J., J. Ahnström, and A. C. Weibull. 2005. The effects of organic agriculture on biodiversity and abundance: A meta-analysis. J. Appl. Ecol. 42:261–269. doi:10.1111/j.1365-2664.2005.01005.x

Berger, W. H. and F. L. Parker. 1970. Diversity of planktonic foraminifera in deep-sea sediments. Science 168:1345–1347. doi:10.1126/science.168.3937.1345

Catalogue of Life in Taiwan (TaiCOL). 2025. Coccinellidae. https://taicol.tw/zh-hant/taxon/t0005668 (visit on 11/9/2025) (in Chinese)

Chen, K. L., W. H. Kong, C. C. Chen, and J. L. Liou. 2021. Evaluating benefits of eco-agriculture: The cases of farms along Taiwan's East Coast in Yilan and Hualien. Sustainability 13:10889. doi:10.3390/su131910889

Chouangthavy, B. and Y. Fourcade. 2024. Organic farming drives higher diversity of beetles, with more predators and less pests. Research Square. doi:10.21203/rs.3.rs-4586391/v1

Chouangthavy, B., S. Sanguansub, and A. Das. 2021. Sustainable organic farming supports diversity

of Coleopteran beetles as a good indicator taxon: A case study from central Lao PDR. Org. Agric. 11:615–624. doi:10.1007/s13165-021-00367-x

Cohen, J. 1988. Statistical Power Analysis for the Behavioral Sciences. 2nd ed. Routledge. New York, NY. 567 pp. doi:10.4324/9780203771587

Crowder, D. W., T. D. Northfield, M. R. Strand, and W. E. Snyder. 2010. Organic agriculture promotes evenness and natural pest control. Nature 466:109–112. doi:10.1038/nature09183

de Paula Malheiros, K., M. V. de Oliveira Santana, C. H. Marchiori, and É. M. de Melo. 2025. The importance of ladybugs (Coleoptera: Coccinellidae) for agriculture and biodiversity in Brazil. Middle East Res. J. Microbiol. Biotechnol. 5:15–25. doi:10.36348/merjmb.2025.v05i01.003

Dong, Y. J., W. H. Yang, P. C. Hsu, C. F. Lee, M. J. Tseng, J. Y. Chang, … H. T. Shih. 2023. Differences in lady beetle species composition between conventional and organic farming environments in Taiwan. J. Taiwan Agric. Res. 72:343–358. (in Chinese with English abstract) doi:10.6156/JTAR.202312_72(4).0005

Garnett, S. T., N. D. Burgess, J. E. Fa, Á. Fernández-Llamazares, Z. Molnár, C. J. Robinson, … I. Leiper. 2018. A spatial overview of the global importance of indigenous lands for conservation. Nat. Sustain. 1:369–374. doi:10.1038/s41893-018-0100-6

Giorgi, J. A., N. J. Vandenberg, J. V. McHugh, J. A. Forrester, S. A. Ślipiński, K. B. Miller, … M. F. Whiting. 2009. The evolution of food preferences in Coccinellidae. Biol. Control 51:215–231. doi:10.1016/j.biocontrol.2009.05.019

Grabovska, T., M. Jelínek, and V. Shevchenko. 2021. Effect of organic farming on the ladybird beetle diversity (Coleoptera: Coccinellidae). Agrobiologiâ 1:188–197. doi:10.33245/2310-9270-2021-163-1-188-197

Hsu G. C., J. A. Ou, and C. K. Ho. 2021. Pest consumption by generalist arthropod predators increases with crop stage in both organic and conventional farms. Ecosphere 12:e03625. doi:10.1002/ecs2.3625

Hsu, G. C., J. A. Ou, M. H. Ni, Z. H. Lin, and C. K. Ho. 2025. Generalist predators function as pest specialists: Examining diet composition of spiders and ladybeetles across rice crop stages. J. Appl. Ecol. 62:1889–1901. doi:10.1111/1365-2664.70066

Koch, R. L. 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non-target impacts. J. Insect Sci. 3:32. doi:10.1093/jis/3.1.32

Kundoo, A. A. and A. A. Khan. 2017. Coccinellids as biological control agents of soft bodied insects: A review. J. Entomol. Zool. Stud. 5:1362–1373.

Letourneau, D. K., I. Armbrecht, B. S. Rivera, J. M. Lerma, E. J. Carmona, M. C. Daza, … A. R. Trujillo. 2011. Does plant diversity benefit agroecosystems? A synthetic review. Ecol. Appl. 21:9–21. doi:10.1890/09-2026.1

Lin, C. M., C. L. Chen, C. M. Tseng, and H. J. Liu. 2024. Discussion on the key success factors of organic operators- A case study of the aboriginal farmers in Hualien area. Bull. Hualien DARES. 42:55–67. (in Chinese with English abstract)

Magurran, A. E. 2004. Measuring Biological Diversity. Blackwell Science. Oxford, UK. 256 pp.

Megha, R. R., A. S. Vastrad, B. C. Kamanna, and N. S. Kulkarni. 2015. Species complex of coccinellids in different crops at Dharwad region. J. Exp. Zool. India 18:931–935.

Obrycki, J. J. and T. J. Kring. 1998. Predaceous Coccinellidae in biological control. Annu. Rev. Entomol. 43:295–321. doi:10.1146/annurev.ento.43.1.295

Patil, P. B. and S. M. Gaikwad. 2019. Diversity and association of ladybird beetles with the agricultural crops. J. Emerg. Technol. Innov. Res. 6:457–459.

Pervez, A., Omkar, and M. M. Harsur. 2020. Coccinellids on crops: Nature's gift for farmers. p.429–460. *in*: Innovative Pest Management Approaches for the 21st Century: Harnessing Automated Unmanned Technologies. (Chakravarthy, A. K. ed.) Springer. Singapore. 519 pp. doi:10.1007/978-981-15-0794-6_21

Poorani, J., R. G. Booth, C. Anuradha, R. G. Gracy, R. Thanigairaj, and R. S. Swathi. 2023. Identity of the 'true' *Micraspis discolor* (Fabricius) (Coleoptera, Coccinellidae) with illustrated diagnostic notes on other *Micraspis* spp. in Indian paddy fields. Zootaxa 5271:446–476. doi:10.11646/zootaxa.5271.3.2

Porcel, M., G. K. S. Andersson, J. Pålsson, and M. Tasin. 2018. Organic management in apple orchards: Higher impacts on biological control than on pollination. J. Appl. Ecol. 55:2779–2789. doi:10.1111/1365-2664.13247

Rada, P., P. Bogusch, P. Pech, J. Pavlíček, J. Rom, and J. Horák. 2023. Active management of urban fruit orchard meadows is important for insect diversity. Ecol. Eng. 186:106833. doi:10.1016/j.ecoleng.2022.106833

Rajan, J. S., S. E. Latha, K. S. Madhuri, R. Vijayaraghavendra, and C. H. S. Rao. 2019. Predatory coccinellids diversity in organic vegetable farming systems: Conservation and mass production. J. Entomol. Zool. Stud. 7:1148–1151.

Saqib, H. S. A., L. Sun, G. Pozsgai, P. Liang, M. You, G. M. Gurr, and S. You. 2022. DNA metabarcoding of gut contents reveals key habitat and seasonal drivers of trophic networks involving generalist predators in agricultural landscapes. Pest Manag. Sci. 78:5390–5401. doi:10.1002/ps.7161

Seago, A. E., J. A. Giorgi, J. Li, and A. Ślipiński. 2011. Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. Mol. Phylogenet. Evol. 60:137–151. doi:10.1016/j.ympev.2011.03.015

Shanker, C., L. Chintagunta, S. Muthusamy, S. Vailla, A. Srinivasan, and G. Katti. 2018. Flora surrounding rice fields as a source of alternative prey for coccinellids feeding on the pests of rice. Eur. J. Entomol. 115:364–371. doi:10.14411/eje.2018.036

Shanker, C., M. Mohan, M. Sampathkumar, C. Lydia, and G. Gatti. 2013. Functional significance of *Micraspis discolor* (F.) (Coccinellidae: Coleoptera) in rice ecosystem. J. Appl. Entomol. 137:601–609. doi:10.1111/jen.12035

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47:561–594. doi:10.1146/annurev.ento.47.091201.145240

Tilman, D. 1999. The ecological consequences of changes in biodiversity: A search for general principles. Ecology 80:1455–1474. doi:10.1890/0012-9658(1999)080[1455:TECOCI]2.0.CO;2

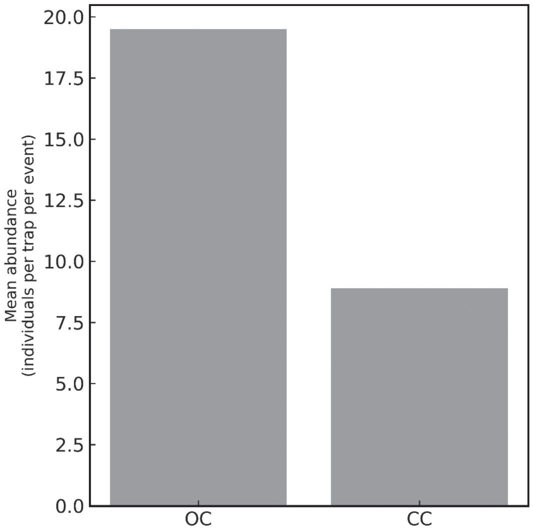
Tscharntke, T., A. M. Klein, A. Kruess, I. Steffan-DewenterI, and C. Thies. 2005. Landscape perspectives on agricultural intensification and biodiversity- Ecosystem service management. Ecol. Lett. 8:857–874. doi:10.1111/j.1461-0248.2005.00782.x

Tscharntke, T., J. M. Tylianakis, T. A. Rand, R. K. Didham, L. Fahrig, P. Batáry, … C. Westphal. 2012. Landscape moderation of biodiversity patterns and processes- Eight hypotheses. Biol. Rev. 87:661–685. doi:10.1111/j.1469-185X.2011.00216.x

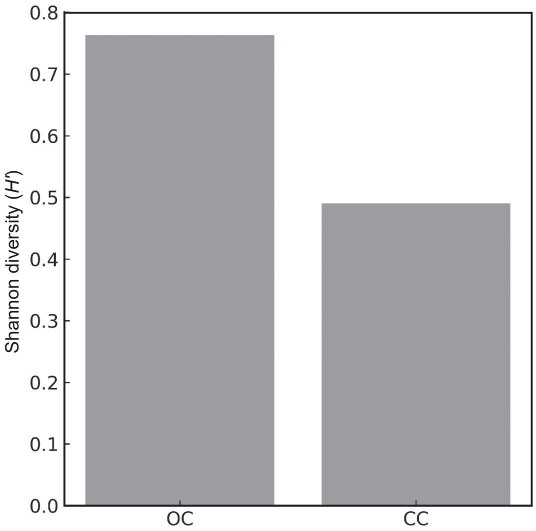
Tuck, S. L., C. Winqvist, F. Mota, J. Ahnström, L. A. Turnbull, and J. Bengtsson. 2014. Land-use intensity and the effects of organic farming on biodiversity: A hierarchical meta-analysis. J. Appl. Ecol. 51:746–755. doi:10.1111/1365-2664.12219

Yen, A. C. and Y. A. Chen. 2014. Sustainable agriculture and indigenous community development: Some experiences in Taiwan. Intl. J. Soc. Sustain. Econ. Soc. Cult. Context 93:85–105. doi:10.18848/2325-1115/CGP/v09i03/55240

Yen, A. C. and Y. A. Chen. 2022. The dilemmas and opportunities for indigenous people in promoting natural farming: A case study of the Tayal Ethnic Group in Jianshi, Taiwan. Taiwan J. Anthrop. 20(2):23–78. (in Chinese with English abstract)



Supplementary Fig. 1. Mean abundance of *Micraspis discolor* (Fabricius, 1798) in organic (OC) and conventional (CC) rice fields during 2022–2024. Bars show the mean number of individuals per trap per sampling event. Values correspond to statistical comparisons presented in Table 3.



Supplementary Fig. 2. Shannon diversity index (H') of lady beetle communities in organic (OC) and conventional (CC) agricultural fields during 2022–2024. Bars represent mean diversity values per sampling event. Statistical results are reported in Table 5.

東臺灣原住民農業系統中有機與慣行農法對天敵瓢蟲群聚組成與穩定性的影響

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摘要

董耀仁、許北辰、李奇峯、曾美容、石憲宗。2026。東臺灣原住民農業系統中有機與慣行農法對天敵瓢蟲群聚組成與穩定性的影響。台灣農業研究 75(2):231–247。

本研究旨在盤點花蓮地區原住民農耕系統中之瓢蟲資源，並評估不同農法對其群聚組成與年際穩定性的影響。2022–2024 年間，於玉里鎮織羅部落之有機 (organic; OC) 與慣行 (conventional; CC) 水稻田，以及瑞穗鄉屋拉力部落之文旦果園進行田野調查，採用黃色黏蟲紙進行瓢蟲標本收集。結果於水稻田共記錄 29 種瓢蟲，以橙瓢蟲 (*Micraspis discolor* (Fabricius, 1798)) 為優勢種；果園則記錄 31 種，以內囊小瓢蟲 (*Scymnus yangi* Yu et Pang, 1993) 與臺南方瓢蟲 (*Sasajiscymnus tainanensis* (Ohta, 1929)) 為優勢種。研究採用 Shannon 多樣性指數 (H') 衡量群聚多樣性、Berger-Parker 優勢度指數 (d) 評估優勢物種比例，以及變異係數 (coefficient of variation; CV) 評估族群穩定性，並以 Mann-Whitney U 檢定比較不同農法間指標之差異 (顯著水準 $P < 0.05$)。結果顯示，在文旦果園中，有機農法顯著提升瓢蟲族群之總數量、多樣性及穩定性；而在水稻田中，有機田區之多樣性未呈現穩定優勢，可能與地區環境條件或周邊土地利用差異有關，仍需進一步探討。整體而言，有機管理在多年生果園系統中有助於提升瓢蟲族群之穩定性與多樣性；但於一年生作物系統 (如水稻) 中的效果則可能因地制宜而異。本研究為首度系統性調查花蓮原鄉農地瓢蟲群聚組成，提供生物防治策略與東部永續農業政策擬定之基礎資料。

關鍵詞：原住民農田、有機農法、慣行農法、天敵瓢蟲、多樣性與穩定性。

投稿日期：2025 年 8 月 15 日；接受日期：2026 年 1 月 28 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):249–263 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0006

Research Article

Susceptibility of Deltamethrin-Resistant *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) to Alternative Insecticides

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Abstract

Feng, W. B., C. Y. Lee, and M. C. Yao. 2026. Susceptibility of deltamethrin-resistant *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) to alternative insecticides. J. Taiwan Agric. Res. 75(2):249–263.

The lesser grain borer, *Rhyzopertha dominica* (Fabricius), is a major pest of stored paddy rice in Taiwan. Deltamethrin has been the primary insecticide recommended for rice-mixing applications in warehouses since 2002. However, its long-term use has led to widespread resistance in field populations, particularly in central and southern Taiwan. To identify potential alternative insecticides, this study evaluated the susceptibility of 20 field-collected *R. dominica* populations with varying levels of deltamethrin resistance to six insecticides representing four different modes of action: 1B organophosphates- malathion and phoxim; 3A pyrethroids- permethrin and phenothrin; 5 spinosyns-spinosad; and 6 avermectins & milbemycins- abamectin. Laboratory bioassays using the rice-mixing method revealed that most populations remained susceptible to malathion, phoxim, spinosad, and abamectin compared to the laboratory population. In contrast, all tested populations showed high resistance to phenothrin. Although some populations showed only moderate resistance to permethrin compared to the laboratory population, they demonstrated extremely high LC_{50} values exceeding 10,000 μg mL^{-1}, indicating limited field utility. These findings suggest that malathion, phoxim, spinosad, and abamectin show potential as alternative insecticides for managing deltamethrin-resistant *R. dominica* in paddy rice warehouses. Field trials are required to validate their efficacy, determine appropriate operational dosages under practical conditions to support future insecticide registration, as well as to establish a science-based insecticide rotation strategy in paddy rice warehouses in Taiwan.

Key words: *Rhyzopertha dominica*, Alternative insecticides, Lethal concentration 50%, Resistance ratio, Level of insecticide resistance.

INTRODUCTION

Rice (*Oryza sativa* Linnaeus) is the third most important staple crop worldwide, following wheat and corn, with an annual production of approximately 776.5 million tons, serving as a primary source of calories for billions of people (FAO 2024). In Taiwan, rice is generally cultivated twice a year across most regions. A portion of the harvested paddy rice is purchased by the government, dried, and stored in designated warehouses as emergency grain reserves, with a maximum storage period of up to three years. However, Taiwan's geographical location as a subtropical island, coupled with the influence of continental air currents, results in persistently high temperatures and humidity. Moreover, the widespread use of sealed, poorly

Received: September 22, 2025; Accepted: December 18, 2025.
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ventilated paddy rice storage facilities creates conditions, highly conducive to the reproduction of stored-product pests. Consequently, paddy rice during storage is frequently infested by several stored-product pests, including *Sitophilus oryzae* (Linnaeus), *Rhyzopertha dominica* (Fabricius), *Cryptolestes ferrugineus* (Stephens), *Oryzaephilus surinamensis* (Linnaeus), and *Lophocateres pusillus* (Klug). These infestations cause substantial quantitative and qualitative losses in paddy rice, with *R. dominica* being the most destructive species (Yao *et al*. 2019, 2022).

R. dominica (Coleoptera: Bostrichidae), commonly known as the lesser grain borer, is a primary pest of cereal-stored products worldwide, particularly in tropical and subtropical regions. It often causes economic losses in maize, rice, wheat and other cereals during storage (Edde 2012). Both larvae and adults are capable of biting the husk of paddy rice and feeding on the endosperm within. This feeding activity results in the fragmentation of paddy rice into powder, which serves as a food source for secondary stored-product pests such as *C. ferrugineus*, *O. surinamensis*, and *Ahasverus advena* (Waltl), thereby exacerbating damage and increasing the risk of grain deterioration. Furthermore, the strong flight ability and phototactic behavior of *R. dominica* often disturb residents living near warehouses, especially between 16:00 and 18:00 (Yao *et al*. 2022), making it not only an economic pest but also a nuisance pest.

Current control methods for stored-product pests rely heavily on insecticide application, which is considered the most effective method for quickly reducing pest populations below the economic injury level (EIL) (Edde 2012). Organophosphates and pyrethroids were the primary insecticides used to control stored-product pests in stored cereals, with deltamethrin being one of the most commonly applied compounds. However, the long-term use of insecticides with a single mode of action may lead to the development of resistance, particularly in pests with short life cycles. Between 1995 and 2012, four species of stored-product pests- *R. dominica*, *S. granarius* (Linnaeus), *S. zeamais* (Motschulsky) and *Tribolium castaneum* (Herbst) were reported to have developed resistance to deltamethrin (Boyer *et al*. 2012). Subsequently, deltamethrin-resistant *R. dominica* populations have been progressively documented in various countries, including Australia (Daglish & Nayak 2018), Brazil (Lorini & Galley 1999), Colombia (Ortega *et al*. 2021), India (Ramesh Babu *et al*. 2017a) and Taiwan (Chen & Chen 2013; Feng *et al*. 2025). In Taiwan, deltamethrin has been the recommended insecticide for controlling stored-product pests in paddy rice warehouses since 2002 (Yao & Lo 2000). Resistance of *R. dominica* to deltamethrin in Taiwan was first reported by Chen & Chen (2013), with the Nantou population exhibiting a resistance ratio of 63.8-fold with an LC_{50} value of 1.276 mg kg^{-1}. After a 12-year interval, Feng *et al*. (2025) reported that 52.4% of the field-collected *R. dominica* populations exhibited high resistance to deltamethrin, with the highest resistance ratio exceeding 117.65-fold. Notably, the proportion of highly deltamethrin-resistant *R. dominica* populations was particularly high in central and southern Taiwan, reaching 60% and 100% respectively (Feng *et al*. 2025). These findings highlight the escalating severity of deltamethrin resistance in *R. dominica*, underscoring the urgent need for effective alternative insecticides to sustain effective pest control and minimize postharvest losses during paddy rice storage.

In this study, the susceptibility of 20 field-collected *R. dominica* populations to six insecticides with four different modes of action, previously described as potential alternative insecticides by Feng *et al*. (2025), was evaluated. The findings aim to provide a scientific reference for selecting alternative insecticides to replace deltamethrin for the control of *R. dominica* in paddy rice warehouses in Taiwan.

MATERIALS AND METHODS

Insect collection and rearing

One susceptible population of *R. dominica*, maintained in the laboratory for over 10 years

without exposure to any insecticides, and 20 field populations were used in this study. Field populations were collected from paddy rice warehouses across Taiwan between September 2022 and October 2023. Detailed information on collection locations is provided in Feng *et al.* (2025).

All populations were reared in plastic containers (diameter: 12 cm, height: 5 cm) with oatmeal provided as their food source. Colonies were maintained in the Laboratory of Integrated Pest Management, Applied Zoology Division, Taiwan Agricultural Research Institute, under controlled environmental conditions of 27 ± 1℃ and 70 ± 5% relative humidity (RH), with continuous darkness.

Insecticides

Technical-grade insecticides were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and included the following active ingredients: 1B organophosphates- malathion (98.60%) and phoxim (97.26%); 3A pyrethroids- permethrin (98.10%) and phenothrin (94.50%); 5 spinosyns- spinosad (93.92%); and 6 avermectins & milbemycins- abamectin (95.69%). Each insecticide was diluted to the desired concentration with acetone and used in subsequent insecticide susceptibility bioassays.

Insecticide susceptibility bioassays

The rice-mixing method was employed to simulate the practical application of insecticide dustable powder, which is the recommended formulation for managing stored-product pests in paddy rice in Taiwan (Yao & Lo 2000). Erlenmeyer flasks (diameter: 5 cm, height: 9 cm) were prepared by first adding 0.1 g of talcum powder, followed by application of 1 mL of insecticide solution at the designated concentration onto the bottom of the flask. The mixture of talcum powder and insecticide solution was left to air-dry under controlled conditions (27 ± 1℃, 70 ± 5% RH) for 3 h to ensure complete evaporation of acetone. After drying, 10 g of paddy rice grains (cultivar: 'Tainan No. 11'; the predominant cultivar cultivated in Taiwan) were introduced into the Erlenmeyer flask and thoroughly mixed with the insecticide-treated talcum powder using a Vortex mixer (Scientific Industries, Inc., New York, NY, USA). Subsequently, thirty unsexed *R. dominica* (F_2–F_3 generation, 2–3 wk old) adults were placed into each flask to initiate the bioassay. Each population was exposed to five concentrations of each insecticide, while the control group was treated with acetone alone. Each concentration was tested in triplicate. All treatments were kept at 27 ± 1℃, 70 ± 5% RH, and a 12 : 12 h (L : D) photoperiod for 24 h. Mortality was evaluated after 24 h, and insects showing no response to touch were considered dead.

Data analysis

Mortality data were analyzed using Probit analysis in the SPSS software (Version 23). The lethal concentration required to cause 50% mortality (LC_{50}), along with its 95% fiducial limits (FL) and chi-squared (χ^2) values, were obtained for each population and insecticide. Resistance ratios (RR) of each population to each insecticide were calculated from the LC_{50} of each population divided by that of the laboratory population. If the mortality of the population was less than 50% at the highest tested insecticide concentration (10,000 μg mL^{-1}), the LC_{50} was recorded as > 10,000 μg mL^{-1}. Based on RR values, resistance levels were classified as follows: low (RR ≤ 10), moderate (10 < RR ≤ 40), high (40 < RR ≤ 160), and extremely high (RR > 160) (Hayashi 1983).

RESULTS

1B Organophosphates: malathion and phoxim

The LC_{50} and RR values of *R. dominica* in response to malathion are shown in Table 1. The Toucheng population was most susceptible to malathion, with an LC_{50} of 141 μg mL^{-1}. All tested populations were susceptible to malathion, displaying LC_{50} values between 141

Table 1. Susceptibility, resistance ratio (RR), and resistance level of *Rhyzopertha dominica* populations to malathion.

	Malathion (1B organophosphates[z])						Deltamethrin (3A pyrethroids)[y]	
Population	N[x]	LC_{50} (95% FL[w]) μg mL^{-1}	Slope	χ^2 (*df*)	RR[v]	Resistance level[u]	RR	Resistance level
Laboratory	450	213 (164–287)	1.13	10.6 (13)	1.00	Low	1.00	Low
Northern								
Yangmei	450	917 (629–1,410)	0.98	27.3 (13)	4.31	Low	6.38	Low
Tongxiao	450	505 (340–875)	1.22	23.5 (13)	2.37	Low	7.60	Low
Tongluo	450	1,368 (1,064–1,864)	1.42	14.0 (13)	6.42	Low	10.31	Moderate
Yuanli	450	523 (345–935)	0.82	15.7 (13)	2.46	Low	48.53	High
Central								
Houli	450	433 (306–680)	0.94	7.8 (13)	2.03	Low	15.19	Moderate
Wufeng	450	262 (203–347)	1.34	3.4 (13)	1.23	Low	> 117.65	High
Shengang	450	507 (399–681)	1.57	5.8 (13)	2.38	Low	9.72	Low
Hemei	450	992 (612–2,014)	0.82	17.6 (13)	4.66	Low	> 117.65	High
Lukang	450	225 (148–363)	1.11	20.8 (13)	1.06	Low	> 117.65	High
Southern								
Minxiong	450	204 (103–551)	0.76	33.5 (13)	0.96	Low	> 117.65	High
Liujiao	450	267 (167–505)	0.37	20.3 (13)	1.25	Low	> 117.65	High
Houbi	450	456 (277–873)	0.82	3.7 (13)	2.14	Low	> 117.65	High
Wandan	450	647 (517–857)	1.89	11.7 (13)	3.04	Low	> 117.65	High
Eastern								
Toucheng	450	141 (88–225)	0.73	10.0 (13)	0.66	Low	2.51	Low
Jiaoxi	450	146 (93–225)	0.76	15.0 (13)	0.69	Low	15.63	Moderate
Dongshan	450	869 (696–1,165)	1.96	6.2 (13)	4.08	Low	> 117.65	High
Yilan	450	1,948 (1,702–2,270)	1.14	39.3 (13)	9.15	Low	> 117.65	High
Sanxing	450	891 (620–1,564)	1.33	9.5 (13)	4.18	Low	> 117.65	High
Changbin	450	366 (176–1,204)	0.88	29.1 (13)	1.72	Low	1.27	Low
Taitung	450	729 (467–1,393)	0.76	21.7 (13)	3.42	Low	13.20	Moderate

[z] The insecticide mode of action was classified by the Insecticide Resistance Action Committee (IRAC).
[y] The RR values and resistance levels of *R. dominica* to deltamethrin were compiled from Feng *et al.* (2025).
[x] Number of *R. dominica* adults tested.
[w] Estimated lethal concentration values with fiducial limits (FL).
[v] Resistance ratio (RR) = LC_{50} of each population divided by LC_{50} of laboratory population.
[u] Level of resistance was classified as low for $RR \leq 10$, moderate for $10 < RR \leq 40$, high for $40 < RR \leq 160$ and extremely high for $RR > 160$.

and 1,948 μg mL^{-1} and RR values from 0.66- to 9.15-fold relative to the laboratory population. All tested populations with moderate to high resistance to deltamethrin remained susceptible to malathion compared with the laboratory population.

The LC_{50} and RR values of *R. dominica* for phoxim are listed in Table 2. The Toucheng and Yilan populations were the most susceptible to phoxim among the tested populations, with LC_{50} of 6 μg mL^{-1}. All tested populations exhibited susceptibility to phoxim, with LC_{50} values ranging from 6 to 100 μg mL^{-1} and RR values ranging from 0.27- to 4.55-fold compared with the laboratory population. All tested populations exhibiting moderate to high resistance to deltamethrin remained susceptible to phoxim, and those with low deltamethrin resistance were likewise generally susceptible when compared with the laboratory population.

3A pyrethroids: permethrin and phenothrin

The LC_{50} and RR values for *R. dominica* exposed to permethrin are detailed in Table 3. The Changbin population exhibited the highest susceptibility to permethrin among the tested populations, with an LC_{50} of 60 μg mL^{-1}. In contrast, the Wufeng, Hemei, Wandan, Jiaoxi, and Yilan populations showed moderate levels of resistance, each with LC_{50} values exceeding 10,000 μg mL^{-1} and RR values greater than 15.17-fold relative to the laboratory population. Most tested populations with high deltamethrin resistance were susceptible to permethrin when compared with the laboratory population. The Wufeng, Hemei, Wandan, Jiaoxi, and Yilan populations, all of which exhibited moderate to high deltamethrin resistance, consistently showed moderate resistance to permethrin.

The LC_{50} and RR values for *R. dominica* with phenothrin are shown in Table 4. The laboratory population exhibited the highest susceptibility to phenothrin, with an LC_{50} value of 130 μg mL^{-1}. However, all tested populations showed high resistance to phenothrin, regardless of their deltamethrin resistance level. All tested populations had LC_{50} values exceeding 10,000 μg mL^{-1} and RR values above 76.92-fold compared to the laboratory population.

5 spinosyns: spinosad

The LC_{50} and RR values of *R. dominica* for spinosad are presented in Table 5. The Wufeng population exhibited the highest susceptibility to spinosad, with an LC_{50} of 1.0 μg mL^{-1}. All tested populations was susceptibile to spinosad, with the RR values ranging from 0.10- to 1.90-fold compared with the laboratory population. All tested populations with moderate to high resistance to deltamethrin remained susceptible to spinosad, and those with low deltamethrin resistance were also susceptible when compared with the laboratory population.

6 avermectins & milbemycins: abamectin

The LC_{50} and RR values of *R. dominica* for abamectin are presented in Table 6. The laboratory population exhibited the highest susceptibility to abamectin, with an LC_{50} value of 339 μg mL^{-1}. Regardless of their resistance levels to deltamethrin, all tested *R. dominica* populations showed low resistance to abamectin, with LC_{50} values ranging from 543 to 1,936 μg mL^{-1} and RR values between 1.60- and 5.71-fold compared to the laboratory population.

DISCUSSION

In this study, the susceptibility of *R. dominica* populations with varying levels of resistance to deltamethrin was evaluated against six insecticides representing four different modes of action. Among the most susceptible populations for each insecticide, spinosad required the lowest concentration to achieve an LC_{50} of 1 μg mL^{-1}. Based on LC_{50}, the insecticidal toxicity ranking was as follows: spinosad (1 μg mL^{-1}) > phoxim (6 μg mL^{-1}) > permethrin (60 μg mL^{-1}) > phenothrin (130 μg mL^{-1}) > malathion (141 μg mL^{-1}) > abamectin (339 μg mL^{-1}). Although LC_{50} values varied across insecticides, toxicity ranking alone is insufficient to determine their suitability for warehouse application. The susceptibility of

Table 2. Susceptibility, resistance ratio, and resistance level of *Rhyzopertha dominica* populations to phoxim.

	Phoxim (1B organophosphates[z])						Deltamethrin (3A pyrethroids)[y]	
Population	N[x]	LC_{50} (95% FL[w]) μg mL^{-1}	Slope	χ^2 (*df*)	RR[v]	Resistance level[u]	RR	Resistance level
Laboratory	450	22 (15–34)	1.30	24.7 (13)	1.00	Low	1.00	Low
Northern								
Yangmei	450	25 (16–42)	0.70	15.1 (13)	1.14	Low	6.38	Low
Tongxiao	450	100 (46–523)	0.67	25.5 (13)	4.55	Low	7.60	Low
Tongluo	450	7 (4–13)	1.88	21.1 (13)	0.32	Low	10.31	Moderate
Yuanli	450	18 (10–34)	1.30	61.3 (13)	0.82	Low	48.53	High
Central								
Houli	450	7 (5–11)	1.49	40.7 (13)	0.32	Low	15.19	Moderate
Wufeng	450	34 (20–76)	1.15	49.2 (13)	1.55	Low	> 117.65	High
Shengang	450	56 (35–109)	0.74	6.7 (13)	2.55	Low	9.72	Low
Hemei	450	23 (15–53)	1.36	21.6 (13)	1.05	Low	> 117.65	High
Lukang	450	31 (23–42)	1.22	18.8 (13)	1.41	Low	> 117.65	High
Southern								
Minxiong	450	33 (21–67)	1.01	14.5 (13)	1.50	Low	> 117.65	High
Liujiao	450	59 (22–69)	0.86	20.0 (13)	2.68	Low	> 117.65	High
Houbi	450	16 (11–24)	1.51	45.4 (13)	0.73	Low	> 117.65	High
Wandan	450	28 (21–40)	1.05	20.2 (13)	1.27	Low	> 117.65	High
Eastern								
Toucheng	450	6 (3–9)	1.23	31.2 (13)	0.27	Low	2.51	Low
Jiaoxi	450	15 (12–21)	1.02	22.3 (13)	0.68	Low	15.63	Moderate
Dongshan	450	14 (8–28)	0.27	29.3 (13)	0.64	Low	> 117.65	High
Yilan	450	6 (2–8)	1.10	27.1 (13)	0.27	Low	> 117.65	High
Sanxing	450	13 (9–20)	1.23	30.4 (13)	0.59	Low	> 117.65	High
Changbin	450	29 (15–82)	0.95	46.5 (13)	1.32	Low	1.27	Low
Taitung	450	99 (69–164)	1.11	10.3 (13)	4.50	Low	13.20	Moderate

[z] The insecticide mode of action was classified by Insecticide Resistance Action Committee (IRAC).
[y] The RR values and resistance levels of *R. dominica* to deltamethrin were compiled from Feng *et al.* (2025).
[x] Number of *R. dominica* adults tested.
[w] Estimated lethal concentration values with fiducial limits (FL).
[v] Resistance ratio (RR) = LC_{50} of each population divided by LC_{50} of laboratory population.
[u] Level of resistance was classified as low for RR ≤ 10, moderate for 10 < RR ≤ 40, high for 40 < RR ≤ 160 and extremely high for RR > 160.

Table 3. Susceptibility, resistance ratio (RR), and resistance level of *Rhyzopertha dominica* populations to permethrin.

	Permethrin (3A pyrethroids[z])						Deltamethrin (3A pyrethroids)[y]	
Population	N[x]	LC_{50} (95% FL[w]) μg mL^{-1}	Slope	χ^2 (*df*)	RR[v]	Resistance level[u]	RR	Resistance level
Laboratory	450	659 (452–1,029)	0.76	6.8 (13)	1.00	Low	1.00	Low
Northern								
Yangmei	450	1,494 (1,169–1,968)	1.26	12.8 (13)	2.27	Low	6.38	Low
Tongxiao	450	1,218 (854–1,806)	1.34	24.0 (13)	1.85	Low	7.60	Low
Tongluo	450	2,678 (1,797–4,786)	1.12	21.8 (13)	4.06	Low	10.31	Moderate
Yuanli	450	2,779 (1,731–5,650)	0.68	17.7 (13)	4.22	Low	48.53	High
Central								
Houli	450	1,963 (1,516–2,669)	0.36	11.0 (13)	2.98	Low	15.19	Moderate
Wufeng	450	> 10,000	-	-	> 15.17	Moderate	> 117.65	High
Shengang	450	3,342 (2,519–4,859)	1.16	13.2 (13)	5.07	Low	9.72	Low
Hemei	450	> 10,000	-	-	> 15.17	Moderate	> 117.65	High
Lukang	450	2,145 (1,342–4,280)	1.19	26.2 (13)	3.25	Low	> 117.65	High
Southern								
Minxiong	450	382 (301–485)	1.28	7.7 (13)	0.58	Low	> 117.65	High
Liujiao	450	1,240 (999–1,557)	1.44	20.5 (13)	1.88	Low	> 117.65	High
Houbi	450	1,498 (1,058–2,247)	0.22	25.0 (13)	2.27	Low	> 117.65	High
Wandan	450	> 10,000	-	-	> 15.17	Moderate	> 117.65	High
Eastern								
Toucheng	450	1,924 (1,521–2,528)	1.29	20.6 (13)	2.92	Low	2.51	Low
Jiaoxi	450	> 10,000	-	-	> 15.17	Moderate	15.63	Moderate
Dongshan	450	2,642 (2,044–3,642)	1.28	21.3 (13)	4.01	Low	>117.65	High
Yilan	450	> 10,000	-	-	> 15.17	Moderate	>117.65	High
Sanxing	450	3,985 (2,867–6,302)	1.16	14.3 (13)	6.05	Low	>117.65	High
Changbin	450	60 (34–94)	1.22	28.5 (13)	0.09	Low	1.27	Low
Taitung	450	329 (124–945)	0.98	75.1 (13)	0.50	Low	13.20	Moderate

[z] The insecticide mode of action was classified by the Insecticide Resistance Action Committee (IRAC).
[y] The RR values and resistance levels of *R. dominica* to deltamethrin were compiled from Feng *et al.* (2025).
[x] Number of *R. dominica* adults tested.
[w] Estimated lethal concentration values with fiducial limits (FL).
[v] Resistance ratio (RR) = LC_{50} of each population divided by LC_{50} of laboratory population.
[u] Level of resistance was classified as low for RR ≤ 10, moderate for 10 < RR ≤ 40, high for 40 < RR ≤ 160 and extremely high for RR > 160.

Table 4. Susceptibility, resistance ratio (RR), and resistance level of *Rhyzopertha dominica* populations to phenothrin.

	Phenothrin (3A pyrethroids[z])						Deltamethrin (3A pyrethroids)[y]	
Population	N[x]	LC_{50} (95% FL[w]) μg mL^{-1}	Slope	χ^2 (*df*)	RR[v]	Resistance level[u]	RR	Resistance level
Laboratory	450	130 (100–173)	1.17	16.8 (13)	1.00	Low	1.00	Low
Northern								
Yangmei	450	> 10,000	-	-	> 76.92	High	6.38	Low
Tongxiao	450	> 10,000	-	-	> 76.92	High	7.60	Low
Tongluo	450	> 10,000	-	-	> 76.92	High	10.31	Moderate
Yuanli	450	> 10,000	-	-	> 76.92	High	48.53	High
Central								
Houli	450	> 10,000	-	-	> 76.92	High	15.19	Moderate
Wufeng	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Shengang	450	> 10,000	-	-	> 76.92	High	9.72	Low
Hemei	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Lukang	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Southern								
Minxiong	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Liujiao	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Houbi	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Wandan	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Eastern								
Toucheng	450	> 10,000	-	-	> 76.92	High	2.51	Low
Jiaoxi	450	> 10,000	-	-	> 76.92	High	15.63	Moderate
Dongshan	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Yilan	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Sanxing	450	> 10,000	-	-	> 76.92	High	> 117.65	High
Changbin	450	> 10,000	-	-	> 76.92	High	1.27	Low
Taitung	450	> 10,000	-	-	> 76.92	High	13.20	Moderate

[z] The insecticide mode of action was classified by the Insecticide Resistance Action Committee (IRAC).
[y] The RR values and resistance levels of *R. dominica* to deltamethrin were compiled from Feng *et al*. (2025).
[x] Number of *R. dominica* adults tested.
[w] Estimated lethal concentration values with fiducial limits (FL).
[v] Resistance ratio (RR) = LC_{50} of each population divided by LC_{50} of laboratory population.
[u] Level of resistance was classified as low for RR ≤ 10, moderate for 10 < RR ≤ 40, high for 40 < RR ≤ 160 and extremely high for RR > 160.

Table 5. Susceptibility, resistance ratio (RR), and resistance level of *Rhyzopertha dominica* populations to spinosad.

	Spinosad (5 spinosyns[z])						Deltamethrin (3A pyrethroids)[y]	
Population	N[x]	LC_{50} (95% FL[w]) μg mL^{-1}	Slope	χ^2 (*df*)	RR[v]	Resistance level[u]	RR	Resistance level
Laboratory	450	10.2 (5.1–17.6)	0.79	36.2 (13)	1.00	Low	1.00	Low
Northern								
Yangmei	450	5.3 (2.4–12.9)	0.73	39.5 (13)	0.52	Low	6.38	Low
Tongxiao	450	3.9 (2.6–5.7)	1.30	31.1 (13)	0.38	Low	7.60	Low
Tongluo	450	4.0 (2.1–7.9)	1.03	45.1 (13)	0.39	Low	10.31	Moderate
Yuanli	450	6.8 (4.1–11.7)	1.03	32.8 (13)	0.67	Low	48.53	High
Central								
Houli	450	1.2 (0.6–2.2)	1.03	30.4 (13)	0.12	Low	15.19	Moderate
Wufeng	450	1.0 (0.7–1.5)	0.88	17.1 (13)	0.10	Low	> 117.65	High
Shengang	450	5.3 (4.1–6.9)	1.17	15.8 (13)	0.52	Low	9.72	Low
Hemei	450	11.4 (7.6–18.6)	0.68	20.0 (13)	1.12	Low	> 117.65	High
Lukang	450	17.3 (8.6–50.8)	0.91	62.0 (13)	1.70	Low	> 117.65	High
Southern								
Minxiong	450	9.6 (5.5–19.4)	0.80	23.5 (13)	0.94	Low	> 117.65	High
Liujiao	450	19.4 (11.8–37.4)	0.62	26.2 (13)	1.90	Low	> 117.65	High
Houbi	450	5.8 (3.7–9.2)	1.22	27.4 (13)	0.57	Low	> 117.65	High
Wandan	450	11.2 (6.4–22.8)	0.96	32.9 (13)	1.10	Low	> 117.65	High
Eastern								
Toucheng	450	4.5 (3.1–6.5)	1.42	29.6 (13)	0.44	Low	2.51	Low
Jiaoxi	450	10.8 (4.9–33.4)	0.74	39.4 (13)	1.06	Low	15.63	Moderate
Dongshan	450	5.5 (2.6–12.6)	0.85	40.3 (13)	0.54	Low	> 117.65	High
Yilan	450	8.7 (6.5–11.9)	0.99	15.9 (13)	0.85	Low	> 117.65	High
Sanxing	450	3.0 (1.6–5.7)	0.95	37.8 (13)	0.29	Low	> 117.65	High
Changbin	450	16.5 (8.8–39.8)	0.62	21.4 (13)	1.62	Low	1.27	Low
Taitung	450	4.1 (2.7–6.1)	0.73	24.4 (13)	0.40	Low	13.20	Moderate

[z] The insecticide mode of action was classified by the Insecticide Resistance Action Committee (IRAC).
[y] The RR values and resistance levels of *R. dominica* to deltamethrin were compiled from Feng *et al.* (2025).
[x] Number of *R. dominica* adults tested.
[w] Estimated lethal concentration values with fiducial limits (FL).
[v] Resistance ratio (RR) = LC_{50} of each population divided by LC_{50} of laboratory population.
[u] Level of resistance was classified as low for RR ≤ 10, moderate for 10 < RR ≤ 40, high for 40 < RR ≤ 160 and extremely high for RR > 160.

Table 6. Susceptibility, resistance ratio (RR), and resistance level of *Rhyzopertha dominica* populations to abamectin.

Population	Abamectin (6 avermectins & milbemycins[z])						Deltamethrin (3A pyrethroids)[y]	
	N[x]	LC_{50} (95% FL[w]) μg mL^{-1}	Slope	χ^2 (*df*)	RR[v]	Resistance level[u]	RR	Resistance level
Laboratory	450	339 (271–429)	1.56	9.7 (13)	1.00	Low	1.00	Low
Northern								
Yangmei	450	1,036 (828–1,314)	1.26	14.3 (13)	3.06	Low	6.38	Low
Tongxiao	450	1,441 (926–1,597)	1.20	27.6 (13)	4.25	Low	7.60	Low
Tongluo	450	543 (431–658)	0.44	21.9 (13)	1.60	Low	10.31	Moderate
Yuanli	450	961 (782–1,184)	1.41	10.3 (13)	2.83	Low	48.53	High
Central								
Houli	450	762 (640–895)	1.82	5.7 (13)	2.25	Low	15.19	Moderate
Wufeng	450	581 (448–717)	1.38	3.8 (13)	1.71	Low	> 117.65	High
Shengang	450	1,542 (1,117–2,426)	1.48	22.8 (13)	4.55	Low	9.72	Low
Hemei	450	975 (811–1,175)	1.58	15.7 (13)	2.88	Low	> 117.65	High
Lukang	450	840 (690–1,014)	1.56	13.2 (13)	2.48	Low	> 117.65	High
Southern								
Minxiong	450	1,418 (941–2,722)	1.05	24.5 (13)	4.18	Low	> 117.65	High
Liujiao	450	1,791 (1,454–2,356)	0.40	7.1 (13)	5.28	Low	> 117.65	High
Houbi	450	754 (572–961)	1.15	7.2 (13)	2.22	Low	> 117.65	High
Wandan	450	1,798 (1480–2,321)	1.74	11.4 (13)	5.30	Low	> 117.65	High
Eastern								
Toucheng	450	1,454 (1,142–1,986)	1.21	12.5 (13)	4.29	Low	2.51	Low
Jiaoxi	450	915 (723–1,157)	1.24	19.7 (13)	2.70	Low	15.63	Moderate
Dongshan	450	1,910 (1,453–2,863)	1.17	18.0 (13)	5.63	Low	> 117.65	High
Yilan	450	1,391 (625–1,689)	1.10	25.5 (13)	4.10	Low	> 117.65	High
Sanxing	450	853 (702–1,023)	1.67	11.8 (13)	2.52	Low	> 117.65	High
Changbin	450	1,244 (526–1,268)	0.71	39.9 (13)	3.67	Low	1.27	Low
Taitung	450	1,936 (1,536–2,662)	1.38	14.3 (13)	5.71	Low	13.20	Moderate

[z] The insecticide mode of action was classified by the Insecticide Resistance Action Committee (IRAC).
[y] The RR values and resistance levels of *R. dominica* to deltamethrin were compiled from Feng *et al.* (2025).
[x] Number of *R. dominica* adults tested.
[w] Estimated lethal concentration values with fiducial limits (FL).
[v] Resistance ratio (RR) = LC_{50} of each population divided by LC_{50} of laboratory population.
[u] Level of resistance was classified as low for RR ≤ 10, moderate for 10 < RR ≤ 40, high for 40 < RR ≤ 160 and extremely high for RR > 160.

deltamethrin-resistant field populations to each insecticide must also be considered. Despite variability in resistance levels, most tested populations were susceptible to malathion, phoxim, spinosad, and abamectin compared to laboratory population, supporting their potential use as alternative insecticides for managing deltamethrin-resistant *R. dominica* populations.

Malathion was initially recommended in 1971 as a replacement for lindane for rice-mixing applications to control stored-product pests in Taiwan (Lin 1972). However, by 1979, the emergence of malathion resistance in stored-product pests led to its replacement by phoxim as the primary insecticide (Wang & Ku 1981). Although malathion remains on the current list of recommended insecticides, it is no longer used for rice-mixing applications in practice. In this study, most *R. dominica* populations were found to be susceptible to malathion compared with the laboratory population, suggesting a decline in malathion resistance over time. Although five populations (Yangmei, Tongluo, Hemei, Yilan, and Sanxing) still exhibited relatively higher LC_{50} values than the 887 μg mL^{-1} reported for the Kuanyin population by Yao & Lo (1994), the overall trend suggests a substantial reversion toward susceptibility. Nevertheless, according to the current formulation and dilution recommendation, the amount of active ingredient applied is approximately 66.7 μg per 10 g of paddy rice, which represents only 0.47 times the LC_{50} value obtained for the most susceptible population (Toucheng) in this study. Therefore, while reintroducing malathion as a rice-mixing insecticide could be a feasible strategy for managing deltamethrin-resistant *R. dominica* populations, an increase in the application rate would likely be required to achieve effective control.

Phoxim was introduced in 1979 for rice-mixing applications to control stored-product pests in Taiwan. However, its continuous use over 2 decades (1979–1999) led to the development of resistance to phoxim in major stored-product pests such as *R. dominica* and *Sitotroga cerealella* (Olivier) (Kao & Tzeng 1992; Yao & Lo 1994, 1995). In response, deltamethrin replaced phoxim in 2002 as the primary insecticide for use in warehouses. Although phoxim is still listed as a recommended insecticide for empty-warehouse disinfection, it has not been used in rice mixing for over 25 years. This prolonged suspension may have contributed to the observed reduction in phoxim resistance. In this study, all tested populations were more susceptible to phoxim than the laboratory population. The Tongxiao population, the most tolerant assessed, had an LC_{50} that was 0.11-fold lower than that of the Kuanyin population reported by Yao & Lo (1994). This finding indicates a substantial reversion toward susceptibility following long-term non-use. However, as phoxim is currently not included on the list of recommended insecticides for rice-mixing, re-registration may be required to enable its use in stored-product pest management in the future.

Spinosad is currently not listed among the recommended insecticides for use in warehouses in Taiwan. However, it has been considered an effective candidate for controlling deltamethrin-resistant *R. dominica* (Chen & Chen 2013; Ramesh Babu *et al.* 2017b). Chen & Chen (2013) reported that deltamethrin-resistant *R. dominica* populations remained susceptible to spinosad, with a maximum resistance ratio of 2.7 and an LC_{50} of 0.264 mg kg^{-1} observed in the Minshong population in Taiwan. In this study, all field populations exhibited lower resistance to spinosad than the laboratory population. Resistance ratios ranged from 0.10 to 1.90, and the highest LC_{50} value (1.94 mg kg^{-1}) was recorded in the Liujiao population. However, when compared with the Minshong population documented by Chen & Chen (2013), the highest LC_{50} observed in this study represents a 7.35-fold increase, indicating a measurable upward shift in spinosad tolerance. Although spinosad and deltamethrin act through different modes of action, Wang *et al.* (2025) confirmed that there was no cross-resistance between spinosad and other insecticides, including malathion (1B), deltamethrin (3A), imidacloprid

(4A), bensultap (14), and chlorantraniliprole (28). Nonetheless, previous studies have indicated the potential for cross-resistance in Hemiptera and Lepidoptera insects (Ishtiaq *et al*. 2012; Banazeer *et al*. 2019). For example, *Spodoptera exigua* (Hübner) strains selected for deltamethrin resistance exhibited moderate resistance to spinosad (Ishtiaq *et al*. 2012), while *Phenacoccus solenopsis* Tinsley population resistant to spinosad also showed resistance to deltamethrin and profenofos (Banazeer *et al*. 2019). These findings suggest that the inclusion of spinosad in insecticide rotation strategies should be accompanied by resistance monitoring and assessment of potential cross-resistance with 1B organophosphates and 3A pyrethroids.

Abamectin is also currently not listed among the recommended insecticides for use in warehouses in Taiwan. However, its potential efficacy against *R. dominica* on several cereal commodities has been demonstrated in previous studies (Kavallieratos *et al*. 2009; Perišić *et al*. 2020). Kavallieratos *et al*. (2009) reported 73.9% mortality of *R. dominica* after 14 d of exposure to abamectin at an application rate of 0.1 mg kg^{-1} on wheat at 30℃, with mortality increasing to 99.4% when the application rate was raised to 0.5 mg kg^{-1}. Similarly, Perišić *et al*. (2020) found that application rates of 0.5–1 mg kg^{-1} on wheat resulted in 67–85% mortality after 14 d, and that complete mortality was achieved at an application rate as low as 0.25 mg kg^{-1} when the exposure duration was extended to 21 d at 26℃. Although both studies evaluated an application rate of 0.5 mg kg^{-1} on wheat, differences in observed mortality after 14 d of exposure were evident. Such variation likely reflects differences in experimental conditions, including temperature, as well as commodity-related factors such as cereal cultivar. Although abamectin has demonstrated efficacy against *R. dominica* on wheat and other cereal commodities, its effectiveness on paddy rice has not yet been evaluated. Consequently, further studies are required to assess the efficacy and practical applicability of abamectin for controlling *R. dominica* under warehouse conditions.

In contrast to the promising alternative insecticides discussed above, permethrin and phenothrin were unsuitable for controlling deltamethrin-resistant *R. dominica*. For permethrin, although only five populations exhibited moderate resistance relative to the laboratory population, the LC_{50} values of the Wufeng, Hemei, Wandan, Jiaoxi, and Yilan populations exceeded 10,000 mg mL^{-1}. These LC_{50} values represent a more than 26.8-fold increase compared with the Kuanyin population reported by Yao & Lo (1994). When compared with the most susceptible population (Changbin) in this study, these four populations demonstrated more than a 166.67-fold increase in LC_{50}, indicating extremely high levels of resistance. Although permethrin remains listed as a recommended insecticide for empty-warehouse disinfection in Taiwan, the elevated LC_{50} values observed here suggest that its continued use may no longer be effective for managing *R. dominica*.

For phenothrin, all tested populations exhibited high levels of resistance, with resistance ratios exceeding 76.9 when compared to the laboratory population. Phenothrin and deltamethrin both belong to the 3A pyrethroid and share the same mode of action by targeting voltage-gated sodium channels in the insect nervous system. This similarity in mode of action suggests a strong likelihood of cross-resistance among insecticides within this insecticide group. Previous studies have reported that deltamethrin-resistant *R. dominica* populations were concurrently resistant to permethrin (Lorini & Galley 2001), further reinforcing the limited utility of pyrethroids as alternative insecticides for managing deltamethrin-resistant populations.

Insecticide rotation is a critical strategy for managing insecticide-resistant pest populations (Madgwick & Kanitz 2024). However, only two insecticides, deltamethrin and malathion, are currently recommended for rice-mixing

applications to control stored-product pests in Taiwan. Among these, malathion is no longer used in practice due to the historical development of resistance in stored-product pests, thereby limiting the feasibility of rotation-based resistance management in paddy rice warehouses in Taiwan. In this study, all tested *R. dominica* populations exhibited susceptibility to four insecticides- malathion, phoxim, spinosad, and abamectin, when compared with the laboratory population under controlled conditions. Nevertheless, actual warehouse trials are required to further evaluate the efficacy of these insecticides in controlling stored-product pests and to determine the appropriate operational dosages under field conditions. To reduce reliance on insecticides with a single mode of action, it is essential to gradually expand the number of insecticides approved for rice-mixing in warehouses. Given that malathion remains on the recommended list for rice-mixing to control *R. dominica* in paddy rice warehouses in Taiwan, forthcoming evaluations will prioritize testing higher application dosages to determine achievable control performance and compliance with residue standards.

In addition to exploring alternative insecticides, incorporating non-chemical controls such as improved ventilation, light traps, and pheromone traps into pest management programs can further strengthen resistance management. Establishing a science-based, integrated strategy that combines insecticides with multiple modes of action and complementary non-chemical controls, specifically tailored to Taiwan's storage systems, will be crucial for delaying the development of resistance and ensuring the long-term effectiveness of recommended insecticides.

ACKNOWLEDGMENTS

We are grateful to Jin-Xia Li, Gui-Xiang Hong, Shu-Xia Hu, and Chien-Yong Yeh for assisting with the experiment. This study was supported by a grant from the Agriculture and Food Agency, Ministry of Agriculture, Taiwan.

REFERENCES

Banazeer, A., M. B. S. Afzal, M. Ijaz, and S. A. Shad. 2019. Spinosad resistance selected in the laboratory strain of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae): Studies on risk assessment and cross-resistance patterns. Phytoparasitica 47:531–542. doi:10.1007/s12600-019-00753-y

Boyer, S., H. Zhang, and G. Lempérière. 2012. A review of control methods and resistance mechanisms in stored-product insects. Bull. Entomol. Res. 102:213–229. doi:10.1017/S0007485311000654

Chen, C. Y. and M. E. Chen. 2013. Susceptibility of field populations of the lesser grain borer, *Rhyzopertha dominica* (F.), to deltamethrin and spinosad on paddy rice in Taiwan. J. Stored Prod. Res. 55:124–127. doi:10.1016/j.jspr.2013.10.001

Daglish, G. J. and M. K. Nayak. 2018. Prevalence of resistance to deltamethrin in *Rhyzopertha dominica* (F.) in eastern Australia. J. Stored Prod. Res. 78:45–49. doi:10.1016/j.jspr.2018.06.003

Edde, P. A. 2012. A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. J. Stored Prod. Res. 48:1–18. doi:10.1016/j.jspr.2011.08.007

Feng, W. B., C. Y. Lee, and M. C. Yao. 2025. Deltamethrin resistance in field populations of *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) collected from paddy rice warehouses in Taiwan. J. Taiwan Agric. Res. 74:1–10. doi:10.6156/JTAR.202503_74(1).0001

Food and Agriculture Organization of the United Nations (FAO). 2024. World Food and Agriculture-Statistical Yearbook 2024. FAO. Rome, Italy. 384 pp. doi:10.4060/cd2971en

Hayashi, A. 1983. History, present status and management of insecticide resistance. p.31–53. *in*: Pest Resistance to Pesticides. (Fukami, J., Y. Uesugi, and K. Ishizuka, eds.) Soft Science. Tokyo, Japan. 412 pp. (in Japanese)

Ishtiaq, M., M. A. Saleem, and D. J. Wright. 2012. Stability, cross-resistance and effect of synergists, PBO and DEF, on deltamethrin resistant strain of *Spodoptera exigua* (Lepidoptera: Noctuidae) from Pakistan. Pakistan J. Zool. 44:1677–1682.

Kao, S. S. and C. C. Tzeng. 1992. A survey of the susceptibility of rice moth (*Corcyra cephalonica*) and Angoumois moth (*Sitotroga cerealella*) to

malathion and phoxim. Chinese J. Entomol. 12:239–245. (in Chinese with English abstract) doi:10.6660/TESFE.1992030

Kavallieratos, N. G., C. G. Athanassiou, B. J. Vayias, S. B. Mihail, and Ž. Tomanović. 2009. Insecticidal efficacy of abamectin against three stored-product insect pests: Influence of dose rate, temperature, commodity, and exposure interval. J. Econ. Entomol. 102:1352–1359. doi:10.1603/029.102.0363

Lin, T. 1972. Determination of rice weevil (*Sitophilus oryzae* Linne) resistance to insecticides and the development of substitutive chemicals. Taiwan Agric. Quarterly 8:115–123. (in Chinese with English abstract)

Lorini, I. and D. J. Galley. 1999. Deltamethrin resistance in *Rhyzopertha dominica* (F.)(Coleoptera: Bostrichidae), a pest of stored grain in Brazil. J. Stored Prod. Res. 35:37–45. doi:10.1016/S0022-474X(98)00028-9

Lorini, I. and D. J. Galley. 2001. The cross-resistance spectrum in deltamethrin resistance strains of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Neotrop. Entomol. 30:321–325. doi:10.1590/S1519-566X2001000200018

Madgwick, P. G. and R. Kanitz. 2024. What is the value of rotations to insecticide resistance management? Pest Manag. Sci. 80:1671–1680. doi:10.1002/ps.7939

Ortega, D. S., T. Bacca, A. P. N. Silva, N. A. Canal, and K. Haddi. 2021. Control failure and insecticides resistance in populations of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) from Colombia. J. Stored Prod. Res. 92:101802. doi:10.1016/j.jspr.2021.101802

Perišić, V., V. Perišić, F. Vukajlović, D. Predojević, V. Rajičić, G. Andrić, and P. Kljajić. 2020. Effects of abamectin on lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), infestation on some stored grains. Egypt. J. Biol. Pest Control 30:116. doi:10.1186/s41938-020-00307-z

Ramesh Babu, S., D. V. Sai Ram Kumar, T. Madhumathi, and V. Manoj Kumar. 2017a. Status of insecticide resistance in lesser grain borer, *Rhyzopertha dominica* to malathion and deltamethrin in Andhra Pradesh. J. Entomol. Zool. Stud. 5(4):741–746.

Ramesh Babu, S., D. V. Sai Ram Kumar, C. H. Naga Satya Sri, and T. Madhumathi. 2017b. Evaluation of spinosad against malathion and deltamethrin resistant population of lesser grain borer, *Rhyzopertha dominica* in Andhra Pradesh, India. Intl. J. Curr. Microbiol. Appl. Sci. 6(6):165–171. doi:10.20546/ijcmas.2017.606.020

Wang, S. C. and T. Y. Ku. 1981. Status of maize weevil resistance to insecticides in Taiwan. Chinese J. Entomol. 1:121–122. (in Chinese with English abstract) doi:10.6660/TESFE.1981014

Wang, Y. C., Y. C. Chen, C. Y. Chen, and M. E. Chen. 2025. Resistance reduction and cross-resistance of spinosad-resistant *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and the association between spinosad resistance and maltase activity. Pest Manag. Sci. 81:2840–2846. doi:10.1002/ps.8650

Yao, M. C., C. Y. Lee, H. W. Chiu, W. B. Feng, E. C. Yang, and K. H. Lu. 2022. Efficiency of a novel light-emitting diode (LED) trap for trapping *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in paddy rice storehouses. J. Econ. Entomol. 115:1294–1302. doi:10.1093/jee/toac054

Yao, M. C. and K. C. Lo. 1994. Phoxin resistance in *Rhyzopertha dominica* Fabricius in Taiwan. Chinese J. Entomol. 14:331–341. (in Chinese with English abstract) doi:10.6660/TESFE.1994027

Yao, M. C. and K. C. Lo. 1995. Phoxim resistance in *Sitotroga cerealella* Olivier in Taiwan. J. Agric. Res. China 44:166–173. (in Chinese with English abstract) doi:10.29951/JARC.199506.0008

Yao, M. C. and K. C. Lo. 2000. Evaluation of deltamethrin and phoxim dust mixed with bagged rough rice for control of storage insects. Chinese J. Entomol. 20:255–266. (in Chinese with English abstract) doi:10.6660/TESFE.2000025

Yao, M. C., J. R. Lu, and C. Y. Lee. 2019. Comparison of pest occurrence and damage in Tainan No. 11 and Taichung Sen No. 10 rice varieties during the storage period. Formosan Entomol. 39:13–28. (in Chinese with English abstract) doi:10.6662/TESFE.201902_39(1).002

第滅寧抗藥性穀蠹 (鞘翅目：長蠹蟲科) 對替代殺蟲劑之感受性評估

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摘要

馮文斌、李啟陽、姚美吉。2026。第滅寧抗藥性穀蠹 (鞘翅目：長蠹蟲科) 對替代殺蟲劑之感受性評估。台灣農業研究 75(2):249–263。

穀蠹 (*Rhyzopertha dominica* (Fabricius)) 為臺灣稻穀倉庫中的重要害蟲。自 2002 年起，第滅寧即為倉庫中稻穀混拌處理所推薦使用的主要殺蟲劑，惟長期使用已導致田間族群普遍產生抗性，特別是在臺灣中部與南部地區。為尋找具潛力之替代藥劑，本研究針對 20 個具不同第滅寧抗藥性程度之穀蠹田間族群，評估其對 4 種不同作用機制之 6 種殺蟲劑的感受性，包括 1B 有機磷類：馬拉松與巴賽松；3A 除蟲菊類：百滅寧與撲滅芬；5 賜諾司類：賜諾殺；以及 6 阿維菌素類 & 米爾貝黴素：阿巴汀。透過稻穀混拌法進行之實驗室生物檢定結果顯示，相較於實驗室敏感品系，大多數族群對馬拉松、巴賽松、賜諾殺及阿巴汀保持感受性。相較之下，所有受測族群皆對撲滅芬呈現高度抗性；另雖部分族群相對於實驗室品系僅呈現中度百滅寧抗性，但其半致死濃度值均超過 10,000 μg mL^{-1}，顯示其田間應用價值極為有限。綜合結果顯示，馬拉松、巴賽松、賜諾殺及阿巴汀具有作為第滅寧抗性穀蠹防治替代藥劑之潛力。後續仍需透過田間試驗驗證其實際防治效果，並確立於實際操作條件下的適宜施用劑量，以支持未來登記使用與建立倉庫稻穀殺蟲劑輪用之科學化管理策略。

關鍵詞：穀蠹、替代殺蟲劑、半致死濃度、抗性比、殺蟲劑抗性程度。

投稿日期：2025 年 9 月 22 日；接受日期：2025 年 12 月 18 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):265–280 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0007

Research Article

Insecticide Susceptibility Dynamics and Control Failure Risk of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to Insecticides in Taiwan

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Abstract

Chiang, M. Y., S. C. Chang, H. Shentu, X. Y. Tsai, S. M. Chang, C. Y. Ku, and C. M. Yang. 2026. Insecticide susceptibility dynamics and control failure risk of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to insecticides in Taiwan. J. Taiwan Agric. Res. 75(2):265–280.

Fall armyworm (FAW), *Spodoptera frugiperda*, invaded Taiwan in 2019 and rapidly became a major pest of maize and sorghum. This study evaluated the susceptibility of field populations from different regions and years to various chemical insecticides- including organophosphates, pyrethroids, neonicotinoids, spinosyns, diamides, and insect growth regulators- and two *Bacillus thuringiensis* (Bt) formulations. At the time of invasion, the FAW population exhibited significant differences in susceptibility to the tested insecticides. Based on control failure likelihood (CFL) values at recommended concentrations, spinetoram, flubendiamide, novaluron, and chlorantraniliprole posed a negligible risk of control failure, whereas Bt subsp. *aizawai* (Bta) NB-200 and flucythrinate presented a high risk. Comparisons of FAW populations collected from mainland Taiwan in 2019 and 2023 showed significantly lower LC_{50} values for chlorantraniliprole and novaluron, but a significantly higher LC_{50} for Bta ABTS-1857, in the 2023 populations. In contrast, susceptibility to etofenprox, spinetoram, and Bta NB-200 did not change significantly. After 38 laboratory generations without insecticide exposure, the laboratory-maintained YY progeny showed a marked recovery of susceptibility to flubendiamide and chlorantraniliprole, with LC_{50} values decreasing by 9.76- and 201.91-fold, respectively. These findings highlight the importance of insecticide selection pressure in FAW resistance dynamics and are consistent with integrated pest management approaches that involve insecticide rotation and the temporary withdrawal of compounds showing elevated resistance.

Key words: Fall armyworm, Insecticide resistance, Diamide insecticides, Control failure likelihood, Integrated pest management.

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (Smith, 1797), is a globally significant agricultural pest in the family Noctuidae (Lepidoptera), which is native to the southern regions of North America. Since its first invasion of Africa in 2016, FAW has rapidly spread across Asia and Oceania, posing a serious threat to global food security due to its preference for staple crops such as maize (*Zea mays*), rice (*Oryza sativa*), and sorghum (*Sorghum bicolor*) (Goergen

Received: October 16, 2025; Accepted: December 19, 2025.
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et al. 2016; Montezano *et al*. 2018). Without effective control measures, FAW can reduce maize yields by up to 45% or cause total crop loss (Hruska & Gladstone 1988; Blanco *et al*. 2016). Its rapid invasion is facilitated by strong migratory ability, short life cycle, polyphagy, and high environmental adaptability.

FAW has also damaged maize expressing toxins of *Bacillus thuringiensis* Berliner, 1915 (Bt) in Latin America, where some populations have developed resistance, reducing the effectiveness of transgenic crop-based control strategies (Blanco *et al*. 2016). Chemical insecticides remain the primary control method. However, resistance has been widely reported in both native and invaded regions, affecting organophosphates, synthetic pyrethroids, benzoylureas, Bt toxins, and diamides (Yu 1991; Omoto *et al*. 2016; Gutiérrez-Moreno *et al*. 2019; Boaventura *et al*. 2020). According to the Arthropod Pesticide Resistance Database, FAW has resistance reports involving 47 active ingredients, with a total of 244 resistance cases documented worldwide (MSU 2025). Migration has been suggested as a potential factor influencing the distribution of resistance-related genes (Samanta *et al*. 2023). Therefore, comprehensive resistance monitoring and a deeper understanding of the underlying molecular and biochemical mechanisms are essential for sustainable management.

FAW is highly invasive, with rapid development, high survival, and extreme fecundity. At 25℃, the egg, larval, and pupal stages last approximately 3.0, 16.2, and 8.6 d, respectively, and a single female can lay over 1,600 eggs during a 7.4-d oviposition period (Huang *et al*. 2021). Since it invaded Taiwan in 2019, FAW has become a major threat to agricultural production. Infestations have been reported primarily on maize and sorghum, which together account for over 90% of reported cases. Maize damage is especially severe because larvae shelter within the whorl during vegetative growth and later bore into ears via the silks, reducing insecticide exposure and directly damaging kernels. Following the 2013 "reactivation of fallow land" policy, maize cultivation in Taiwan increased by 1.26-fold relative to the previous year and expanded to 2.22 times the 2012 level by 2023. For this study, Yunlin County, representing about 43.6% of Taiwan's sweet corn area in 2019, was selected as the primary FAW sampling region (Query of crops by cultivation scale, https://agr.afa.gov.tw/afa/afa_frame.jsp).

This study originated from the first author's participation in the early emergency response to FAW invasion into Taiwan, when information on insecticide efficacy and regional susceptibility was limited. The objectives were to evaluate the efficacy of commonly used insecticides against FAW larvae and to compare susceptibility among regional populations. This information provides a scientific basis for insecticide selection, and future resistance monitoring and management.

MATERIALS AND METHODS

Insect populations

FAW populations were collected from four locations on Taiwan's main island and one offshore island (Kinmen) for insecticide susceptibility assays (Fig. 1). The YY population was established in 2019 by pooling approximately 30–50 larvae collected from maize fields in Dongshi Township, Yunlin County (Western Taiwan), together with 5–10 larvae collected from maize fields in Toucheng Township, Yilan County (eastern Taiwan), serving as a representative FAW population for Taiwan. In 2023, the YL population was established by pooling 50–100 larvae from maize fields in Baozhong and Yuanchang Townships, Yunlin County. The KM population was collected from sorghum fields in Jinsha Township, Kinmen County. Sampling locations were plotted in MapInfo Professional 8.5 with base map data obtained from the Ministry of the Interior, Taiwan.

Collected larvae were individually reared under controlled laboratory conditions (25 ± 1℃, 12 h photoperiod, 60 ± 5% relative humidity (RH)). The YY population was maintained on fresh maize leaves for four mon

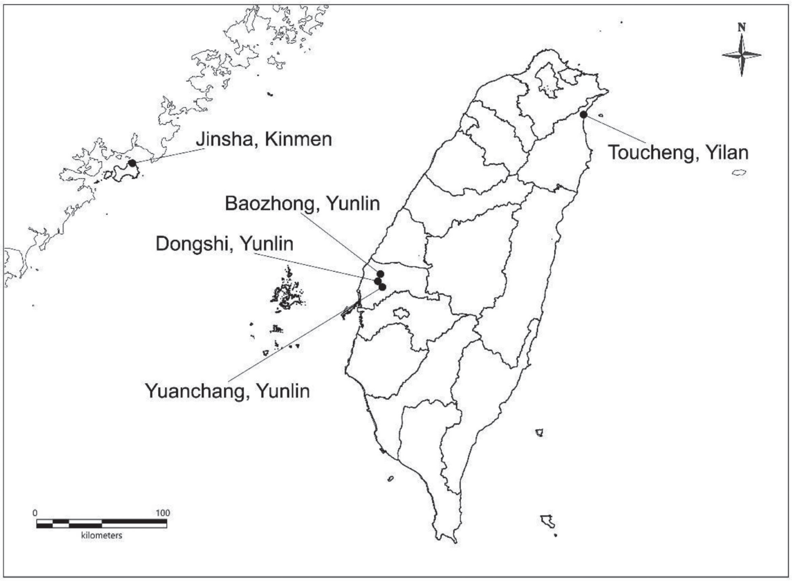

Fig. 1. Collecting locations of *Spodoptera frugiperda* populations in Taiwan and its outlying islands. Map generated with MapInfo Professional 8.5 using administrative boundary data from the Ministry of the Interior, Taiwan.

before switching to an artificial diet modified from Kao (1995), whereas the YL and KM populations were reared on the artificial diet immediately after field collection. Pupae were surface-sterilized in 1.5% sodium hypochlorite solution (Clorox bleach, 5.5–6.0% active ingredient; Clorox Company, Oakland, CA, USA) for 10 min and sexed based on the terminal abdominal segments (Luginbill 1928).

Ten female and ten male pupae were placed in an oviposition cage (diameter 14 cm × height 20 cm) for adult emergence, mating, and egg laying. Adults were supplied with 20% honey solution. At 25℃, the oviposition period of FAW is 7.4 d (Huang *et al*. 2021). To synchronize larval age and maximize egg yield, only egg masses laid 5–7 d after the onset of oviposition were collected. Larvae were initially reared in groups and then individually from the third instar until pupation. Successive generations were maintained using the same procedures.

Insecticides

The insecticides used in this study are listed in Table 1. Selection was based on commercial availability and government-recommended emergency control insecticides for FAW on maize and sorghum in Taiwan (https://faw.aphia.gov.tw/). Malathion, chlorfenapyr, and thiamethoxam were additionally included: malathion and chlorfenapyr are recommended for FAW on other host crops, while thiamethoxam, a systemic insecticide recommended for aphid control on sorghum, may be suitable for controlling FAW larvae concealed within the whorl or near the growing point of maize and sorghum (https://pesticide.aphia.gov.tw/information/).

For bioassays, each insecticide was prepared in distilled water containing 0.03% Triton X-100 (Merck KGaA, Darmstadt, Germany) as a surfactant.

Insecticide toxicity during the early invasion of FAW

1. Leaf-dip bioassay

F_1 and F_2 larvae of the YY population collected in 2019 were used. Due to the limited number of offspring, each insecticide was tested sequentially at three concentrations to rapidly identify effective compounds against

Table 1. Pesticides used in this study.

Insecticides	Active ingredient (a.i.)	Formulations[z]	IRAC MoA group[y]	Dilution fold (Target pest)[x]	Manufacturer
Malathion	50.0%	EC	1B	500× (Noctuid pests on vegetable)	Harvest Chemical
Etofenprox	20.0%	WP	3A	1,500× (FAW on maize and sorghum)	Mitsui Chemicals
Flucythrinate	5.0%	SL	3A	800× (FAW on maize and sorghum)	Chia Tai
Thiamethoxam	25.0%	SG	4A	5,000× (Aphid on sorghum)	Syngenta
Spinetoram	11.7%	SC	5	1,200× (FAW on maize and sorghum)	DOW AgroSciences
Bta NB-200	54.0%	WG	11	1,000× (FAW on maize and sorghum)	Sumitomo Chemical
Bta ABTS-1857[w]	48.1%	WG	11	1,000× (FAW on maize and sorghum)	Sumitomo Chemical
Chlorfenapyr	10.0%	SC	13	1,000× (Noctuid pests on peanut and vegetables)	BASF
Novaluron[x]	10.0%	DC	15	1,500× (FAW on maize and sorghum)	Lanlix Crop Science
Chlorantraniliprole	18.4%	SC	28	2,500× (FAW on maize and sorghum)	FMC Taiwan
Flubendiamide	20.0%	WG	28	3,000× (FAW on maize and sorghum)	Taiwan Nihon Nohyaku

[z] EC: emulsifiable concentrate; WP: wettable powder; SL: soluble liquid; SG: water-soluble granules; SC: suspension concentrate; WG: water-dispersible granules; DC: dustable powder.
[y] IRAC MoA groups are based on the Insecticide Resistance Action Committee mode of action classification.
[x] Dilution fold (target pest): based on the field application rate recommended by the Animal and Plant Health Inspection Agency (APHIA) for each target pest in Taiwan.
[w] *Bacillus thuringiensis* subsp. *aizawai* fermentation products: solids, spores, and insecticidal toxins.

the invasive population, rather than performing a conventional LC_{50} bioassay, which typically requires 5–6 concentrations. Tested insecticides (Table 1) were prepared at 0.5-, 1-, and 2-fold of their respective recommended field application rates for maize pest control in Taiwan, following the simplified resistance monitoring protocol of Cheng *et al*. (1992). Maize leaves (5–9 wk old) grown in netted greenhouses were cut into 5 × 5 cm^2 pieces, dipped in insecticide solutions containing 0.03% Triton X-100 for 15 s, and air-dried on 20 × 20 cm^2 filter paper. Control leaves were dipped only in 0.03% Triton X-100.

Petri dishes (9 cm in diameter) were lined with 7 cm filter paper moistened with 0.8 mL of distilled water. Two treated leaf pieces were placed in each dish with partial overlap, and a total of ten late second-instar larvae were transferred onto the leaves using a soft brush. Larvae were maintained at 25 ± 1℃, with a 12 h photoperiod and 75 ± 5% relative humidity. Mortality was recorded every 24 h for three consecutive days, with four replicates per treatment. Larvae unable to right themselves or showing no normal movement when gently probed were considered dead.

2. Control failure likelihood (CFL)

To assess the risk of control failure due to insecticide resistance, CFL was calculated following Guedes (2017). The CFL was calculated using the following equation:

$$CFL = 100 - [\text{observed mortality (\%)} \times 100] / \text{expected mortality (\%)}$$

The expected mortality for *S. frugiperda* was set at 70% (Pudasaini *et al*. 2022; Ahissou *et al*. 2024). CFL values were determined based on mortality recorded 72 h after treatment at the respective recommended field concentrations. CFL values were classified as low (0–10%), moderate (10–20%), high (20–30%), or extremely high (> 30%) to indicate increasing risk of control failure, and values < 0% were considered to indicate negligible risk.

Spatiotemporal variation in insecticide susceptibility

Susceptibility to seven insecticides, including etofenprox, spinetoram, Bta ABTS-1857, Bta NB-200, novaluron, flubendiamide, and chlorantraniliprole, was evaluated using laboratory-reared progeny of three FAW populations. Flucythrinate was included as a representative pyrethroid insecticide from the emergency control program for FAW in the previous CFL analysis. However, because etofenprox, another pyrethroid, is registered for use on a broader range of crops and has been applied more frequently in agricultural practice in Taiwan, it was selected for the present study to evaluate FAW susceptibility.

The populations tested included YY, collected from Yunlin and Yilan Counties during the initial invasion in 2019. Field populations, YL and KM, were collected in 2023 from Yunlin County and Kinmen County, respectively. YY F_5 was tested in 2019, whereas YY F_{43} and F_{45} were evaluated in 2023 to examine differences among laboratory generations and populations collected in different years.

After collection, all populations were reared under laboratory conditions without further exposure to insecticides. The progeny of YL and KM populations from F_1 to F_8 were evaluated. All bioassays were conducted using the leaf-dip method described above. Each insecticide was tested at six concentrations to generate dose–response curves, and median lethal concentrations (LC_{50}) were estimated using Probit Analysis using MSChart software (Finney 1971; Chi 2024).

Statistical analysis

Susceptibility among FAW populations was compared based on 95% confidence intervals (CIs) of LC_{50} values. Non-overlapping CIs were considered indicative of significant differences between populations (Deshmukh *et al*. 2020; Lira *et al*. 2020).

RESULTS

Insecticide toxicity during the early invasion of FAW

The insecticide susceptibility of the F_1 and F_2 progeny of the YY population collected during the initial FAW invasion in Taiwan in 2019 is summarized in Table 2. The tested insecticides showed marked differences in both speed of action and overall effectiveness against FAW. Spinetoram and chlorfenapyr were the fastest-acting, causing 100% mortality within 24 h at all tested concentrations (0.5-, 1-, and 2-fold recommended field rates for maize). Flubendiamide and chlorantraniliprole were comparatively slower-acting, with 48 h mortality ranging from 70.00–91.67%, reaching near-complete mortality only after 72 h. Novaluron required 72 h to achieve 83.82–100.00% mortality.

Table 2. Toxicity of insecticides to *Spodoptera frugiperda* during the initial invasion in Taiwan 2019.

Insecticide (active ingredient)	Dilution fold[z]	Active ingredient (μg mL^{-1})	Mortality (mean ± *SD*) (%)		
			24 h	48 h	72 h
Control	-		0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spinetoram (11.7%)	2,400×	48.8	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	1,200×	97.5	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	600×	195.0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Chlorfenapyr (10.0%)	2,000×	50.0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	1,000×	100.0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	500×	200.0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Flubendiamide (20.0%)	6,000×	33.3	35.97 ± 9.99	91.67 ± 16.67	94.44 ± 6.42
	3,000×	66.7	34.19 ± 14.06	80.00 ± 16.33	100.00 ± 0.00
	1,500×	133.3	35.00 ± 17.32	82.50 ± 9.57	100.00 ± 0.00
Chlorantraniliprole (18.4%)	5,000×	36.8	5.33 ± 6.05	75.56 ± 14.17	94.44 ± 6.42
	2,500×	73.6	2.80 ± 5.54	82.22 ± 17.08	97.50 ± 10.00
	1,250×	147.2	6.73 ± 5.66	70.00 ± 11.55	97.50 ± 5.00
Novaluron (10.0%)	3,000×	33.3	0.00 ± 0.00	18.06 ± 23.73	83.82 ± 12.95
	1,500×	66.7	7.50 ± 5.00	15.70 ± 12.52	100.00 ± 0.00
	750×	133.3	7.50 ± 9.57	17.50 ± 9.57	97.50 ± 5.00
Malathion (50.0%)	1,000×	500.0	17.50 ± 9.57	30.00 ± 8.16	30.00 ± 8.16
	500×	1,000.0	44.44 ± 22.19	53.89 ± 20.58	53.61 ± 18.06
	250×	2,000.0	72.22 ± 27.31	85.00 ± 17.32	85.00 ± 17.32
Thiamethoxam (25.0%)	10,000×	25.0	7.50 ± 9.57	17.50 ± 28.72	22.50 ± 33.04
	5,000×	50.0	7.04 ± 6.12	10.00 ± 8.16	23.06 ± 4.75
	2,500×	100.0	75.00 ± 23.80	82.50 ± 17.08	82.50 ± 17.08
Bta NB-200[y] (54.0%)	2,000×	270.0	24.32 ± 17.11	29.32 ± 14.21	29.09 ± 13.38
	1,000×	540.0	31.25 ± 10.31	43.75 ± 14.93	51.25 ± 23.94
	500×	1,080.0	53.89 ± 12.53	74.17 ± 17.72	76.67 ± 17.64
Flucythrinate (5.0%)	1,600×	31.3	0.00 ± 0.00	3.13 ± 6.25	13.75 ± 16.01
	800×	62.5	40.65 ± 3.52	43.89 ± 11.28	50.14 ± 19.63
	400×	125.0	35.28 ± 20.62	41.84 ± 21.21	46.94 ± 22.89

[z] Dilution fold: Concentrations represent 0.5-, 1-, and 2-fold of the respective field application rates recommended by Animal and Plant Health Inspection Agency (APHIA).

[y] *Bacillus thuringensis* subsp. *aizawai* NB-200.

In contrast, malathion, thiamethoxam, and Bta NB-200 exhibited lower overall efficacy, with mortality varying depending on dose and exposure time. Malathion and thiamethoxam exhibited relatively rapid initial toxicity, with 72.22 and 75.00% mortality, respectively, at the highest concentration after 24 h, but mortality increased only slightly by 48 h and remained below 85%. Flucythrinate exhibited the lowest efficacy, with a maximum mortality of 50.14% after 72 h across all concentrations. These results highlight clear differences in both the speed of action and overall effectiveness among the tested insecticides.

Based on CFL values at the recommended concentrations, the risk of control failure was negligible for spinetoram, flubendiamide, novaluron, and chlorantraniliprole, whereas Bta NB-200 and flucythrinate presented a high risk of control failure. Malathion, chlorfenapyr, and thiamethoxam were excluded from this analysis, as they are not recommended for FAW control on maize or sorghum (Fig. 2).

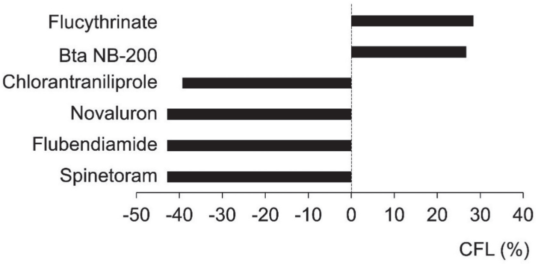

Fig. 2. Control failure likelihood (CFL) for *Spodoptera frugiperda*, evaluated at the recommended field concentrations at 72 h post-treatment during the initial invasion in Taiwan in 2019.

Spatiotemporal variation in insecticide susceptibility

FAW populations collected from different regions and years were compared across successive laboratory generations in the absence of insecticide exposure to examine regional heterogeneity and differences in susceptibility patterns among populations collected in different years. Based on this initial assessment, three FAW populations were selected for bioassays using seven insecticides (Tables 3–9).

No significant differences in LC_{50} values were observed among populations for etofenprox or spinetoram, indicating similar susceptibility to these insecticides (Tables 3–4). For Bta ABTS-1857, the YL population exhibited significantly reduced susceptibility compared with the YY population, whereas no significant differences were detected between YL and KM or between the YY F_5 and F_{45} generations (Table 5). In contrast, Bta NB-200 showed the opposite pattern, with the YL population being significantly more susceptible than YY F_{45} and KM (Table 6). Novaluron also displayed population-specific responses, with YL remaining more susceptible than YY (Table 7).

Clear generational trends were observed for flubendiamide and chlorantraniliprole. Within the YY population collected from the field in 2019, the F_{43} generation exhibited 9.8- and 201.9-fold lower LC_{50} values than the F_5 generation, respectively, indicating substantial recovery of susceptibility after prolonged laboratory rearing without insecticide exposure. For both insecticides, the YL population

Table 3. The susceptibility of different populations of *Spodoptera frugiperda* to etofenprox.

Populations[z]	Year	Generation	LC_{50} (μg mL^{-1})[y]	95% CL of LC_{50} (μg mL^{-1})[x]	Slope ± *SE*	χ^2 (*df*)	Resistance ratio[w]
YY	2019	F_{43}	36.06 a	19.85–64.86	2.70 ± 0.57	2.32 (2)	
YL	2023	F_7	74.25 a	29.07–42.47	1.25 ± 0.19	2.58 (3)	2.06
KM	2023	F_3	41.64 a	33.66–51.46	2.93 ± 0.40	3.45 (4)	1.15

[z] YY: initial invasion population, 2019, maize, Dongshi (Yunlin) & Toucheng (Yilan); YL: 2023, maize, Baozhong & Yuanchang (Yunlin); KM: 2023, sorghum, Jinsha (Kinmen).

[y] LC_{50} values followed by the same letter do not show significant differences based upon non-overlapping confidence limits 95%.

[x] CL: confidence limit.

[w] Resistance ratio: calculated using the YY population as the reference susceptible population.

Table 4. The susceptibility of different populations of *Spodoptera frugiperda* to spinetoram.

Populations[z]	Year	Generation	LC_{50} (μg mL^{-1})[y]	95% CL of LC_{50} (μg mL^{-1})[x]	Slope ± *SE*	χ^2 (*df*)	Resistance ratio[w]
YY	2019	F_{43}	0.070 a	0.055–0.089	3.81 ± 0.62	1.35 (2)	
YL	2023	F_1	0.042 a	0.010–0.182	1.40 ± 0.15	4.04 (2)	0.60
KM	2023	F_6	0.021 a	0.005–0.090	1.64 ± 0.20	5.26 (2)	0.30

[z] YY: initial invasion population, 2019, maize, Dongshi (Yunlin) & Toucheng (Yilan); YL: 2023, maize, Baozhong & Yuanchang (Yunlin); KM: 2023, sorghum, Jinsha (Kinmen).
[y] LC_{50} values followed by the same letter do not show significant differences based upon non-overlapping confidence limits 95%.
[x] CL: confidence limit.
[w] Resistance ratio: calculated using the YY population as the reference susceptible population.

Table 5. The susceptibility of different populations of *Spodoptera frugiperda* to *Bacillus thuringensis* subsp. *aizawai* ABTS-1857.

Populations[z]	Year	Generation	LC_{50} (μg mL^{-1})[y]	95% CL of LC_{50} (μg mL^{-1})[x]	Slope ± *SE*	χ^2 (df)	Resistance ratio[w]
YY	2019	F_{45}	238.12 a	191.15–296.72	2.18 ± 0.24	2.34 (4)	
		F_5	341.84 a	229.43–507.86	1.46 ± 0.26	2.38 (4)	1.44
YL	2023	F_8	957.51 b	554.16–1,657.84	2.79 ± 0.40	3.23 (2)	4.02
KM	2023	F_1	451.87 ab	270.08–753.84	1.97 ± 0.24	3.95 (3)	1.90

[z] YY: initial invasion population, 2019, maize, Dongshi (Yunlin) & Toucheng (Yilan); YL: 2023, maize, Baozhong & Yuanchang (Yunlin); KM: 2023, sorghum, Jinsha (Kinmen).
[y] LC_{50} values followed by the same letter do not show significant differences based upon non-overlapping confidence limits 95%.
[x] CL: confidence limit.
[w] Resistance ratio: calculated using the YY population as the reference susceptible population.

Table 6. The susceptibility of different populations of *Spodoptera frugiperda* to *Bacillus thuringensis* subsp. *aizawai* NB-200.

Populations[z]	Year	Generation	LC_{50} (μg mL^{-1})[y]	95% CL of LC_{50} (μg mL^{-1})[x]	Slope ± *SE*	χ^2 (*df*)	Resistance ratio[w]
YY	2019	F_{45}	308.76 a	218.31–437.05	2.02 ± 0.29	2.66 (3)	
		F_5	246.35 ab	102.17–585.91	0.93 ± 0.28	1.60 (3)	0.80
YL	2023	F_7	157.02 b	120.21–205.15	2.01 ± 0.23	1.12 (3)	0.51
KM	2023	F_1	476.28 a	280.82–809.16	1.70 ± 0.26	1.09 (2)	1.54

[z] YY: initial invasion population, 2019, maize, Dongshi (Yunlin) & Toucheng (Yilan); YL: 2023, maize, Baozhong & Yuanchang (Yunlin); KM: 2023, sorghum, Jinsha (Kinmen).
[y] LC_{50} values followed by the same letter do not show significant differences based upon non-overlapping confidence limits 95%.
[x] CL: confidence limit.
[w] Resistance ratio: calculated using the YY population as the reference susceptible population.

Table 7. The susceptibility of different populations of *Spodoptera frugiperda* to novaluron.

Populations[z]	Year	Generation	LC_{50} (μg mL^{-1})[y]	95% CL of LC_{50} (μg mL^{-1})[x]	Slope ± *SE*	χ^2 (*df*)	Resistance ratio[w]
YY	2019	F_{43}	1.00 a	0.65–1.42	1.50 ± 0.25	0.36 (2)	
YL	2023	F_7	0.22 b	0.12–0.41	1.54 ± 0.19	3.29 (3)	0.22
KM	2023	F_6	0.74 ab	0.37–1.34	1.47 ± 0.17	2.57 (3)	0.74

[z] YY: initial invasion population, 2019, maize, Dongshi (Yunlin) & Toucheng (Yilan); YL: 2023, maize, Baozhong & Yuanchang (Yunlin); KM: 2023, sorghum, Jinsha (Kinmen).
[y] LC_{50} values followed by the same letter do not show significant differences based upon non-overlapping confidence limits 95%.
[x] CL: confidence limit.
[w] Resistance ratio: calculated using the YY population as the reference susceptible population.

generally exhibited the highest LC_{50} values, while KM showed intermediate susceptibility (Tables 8 and 9).

All χ^2 tests were non-significant ($P > 0.05$), confirming an adequate fit of the Probit model and reliability of the LC_{50} estimates. Susceptibility patterns differed among populations depending on insecticide and population origin, and partial recovery was observed over successive laboratory generations.

DISCUSSION

The insecticides evaluated in this study exhibited distinct lethal dynamics against FAW, reflecting their differing modes of action. Malathion, thiamethoxam, and flucythrinate caused rapid mortality, with slight variation among mortality recorded at 24, 48, and 72 h post-treatment, indicating that most of their lethal effects occurred within the first 24 h (Table 2). Malathion inhibits acetylcholinesterase, preventing the degradation of acetylcholine and causing persistent synaptic excitation (Casida & Durkin 2013). Thiamethoxam acts as a nicotinic acetylcholine receptor (nAChR) modulator, leading to overstimulation of neural receptors (Tomizawa & Casida 2005), while flucythrinate functions as a sodium channel modulator, disrupting neuronal depolarization (Soderlund 2012). Despite acting on different neural targets, all three compounds rapidly incapacitate FAW through neurotoxic action (Casida & Durkin 2013; Sparks & Nauen 2015). However, it is important to note that although these compounds exhibit rapid initial toxicity, their overall mortality rates failed to reach the threshold for reliable field control, and some, such as thiamethoxam, are not currently registered for FAW management on maize in Taiwan.

In contrast, flubendiamide, chlorantraniliprole, and Bta NB-200 caused progressively increasing mortality, whereas novaluron required up to 72 h to exceed 80% mortality, reflecting its slower mode of action (Table 2). These mortality patterns correspond to the physiological targets

Table 8. The susceptibility of different populations of *Spodoptera frugiperda* to flubendiamide.

Populations[z]	Year	Generation	LC_{50} (μg mL^{-1})[y]	95% CL of LC_{50} (μg mL^{-1})[x]	Slope ± *SE*	χ^2 (*df*)	Resistance ratio[w]
YY	2019	F_{43}	0.79 b	0.53–1.17	1.38 ± 0.16	1.32 (3)	
		F_5	7.71 a	3.22–17.76	1.73 ± 0.46	0.71 (2)	9.76
YL	2023	F_2	2.85 ab	2.05–3.91	1.65 ± 0.18	2.15 (4)	3.61
KM	2023	F3	1.61 b	0.48–2.79	0.96 ± 0.18	2.33 (3)	2.04

[z] YY: initial invasion population, 2019, maize, Dongshi (Yunlin) & Toucheng (Yilan); YL: 2023, maize, Baozhong & Yuanchang (Yunlin); KM: 2023, sorghum, Jinsha (Kinmen).
[y] LC_{50} values followed by the same letter do not show significant differences based upon non-overlapping confidence limits 95%.
[x] CL: confidence limit.
[w] Resistance ratio: calculated using the YY population as the reference susceptible population.

Table 9. The susceptibility of different populations of *Spodoptera frugiperda* to chlorantraniliprole.

Populations[z]	Year	Generation	LC_{50} (μg mL^{-1})[y]	95% CL of LC_{50} (μg mL^{-1})[x]	Slope ± *SE*	χ^2 (*df*)	Resistance ratio[w]
YY	2019	F_{43}	0.035 b	0.031–0.039	2.01 ± 0.21	0.20 (3)	
		F_5	7.067 a	4.589–10.808	2.87 ± 0.64	1.34 (3)	201.91
YL	2023	F_6	0.585 c	0.407–0.833	4.50 ± 0.58	2.18 (2)	16.71
KM	2023	F_6	0.098 b	0.034–0.321	0.85 ± 0.12	3.87 (3)	2.80

[z] YY: initial invasion population, 2019, maize, Dongshi (Yunlin) & Toucheng (Yilan); YL: 2023, maize, Baozhong & Yuanchang (Yunlin); KM: 2023, sorghum, Jinsha (Kinmen).
[y] LC_{50} values followed by the same letter do not show significant differences based upon non-overlapping confidence limits 95%.
[x] CL: confidence limit.
[w] Resistance ratio: calculated using the YY F_{43} population as the reference susceptible population.

of the compounds. Flubendiamide and chlorantraniliprole disrupt calcium signaling by modulating ryanodine receptors (Sattelle *et al*. 2008). Bt kills FAW through the synergistic action of its Cry and Vip toxins, disrupting the midgut and causing death from septicemia (Gonzalez *et al*. 2008; Sanchis & Bourguet 2008; Pacheco *et al*. 2021; Lazo *et al*. 2025; Chang *et al*. 2026). Novaluron, a chitin synthesis inhibitor, interferes with molting and cuticle formation (Cutler & Scott-Dupree 2007).

Thus, while neurotoxic insecticides provide rapid control, compounds such as diamides, Bt, and novaluron act more slowly. This slower action may permit continued crop feeding during the lag phase. Moreover, evaluation across three concentrations indicated that spinetoram, flubendiamide, chlorantraniliprole, and novaluron maintained good efficacy even at 0.5-fold of the recommended field rate. These findings provide clearer guidance for insecticide rotation decisions and highlight the importance of including these compounds in rotation strategies to support long-term resistance management.

To facilitate rapid resistance detection in invasive FAW populations, this study adopted the simplified resistance monitoring protocol of Cheng *et al*. (1992), in which bioassays were conducted at 0.5-, 1-, and 2-fold of the respective recommended field application rates.

Based on CFL analysis at the recommended field rates, spinetoram, flubendiamide, novaluron, and chlorantraniliprole showed a negligible risk of control failure (CFL < 0%), supporting their expected efficacy under field conditions. In contrast, Bta NB-200 and flucythrinate were associated with a high risk of control failure (CFL 20–30%) at their respective recommended field rates, indicating the need for restricted use (Fig. 2).

From a resistance management perspective, insecticide rotation incorporating compounds with distinct modes of action may help reduce selection pressure on FAW populations. In addition, temporary withdrawal of insecticides a higher risk of control failure could potentially facilitate partial recovery of susceptibility, as observed in laboratory-maintained populations. Continuous susceptibility monitoring is therefore essential for monitoring the diversity of resistance profiles and for informing timely adjustments to FAW management strategies.

Collectively, the present results indicate that spinetoram, flubendiamide, novaluron, and chlorantraniliprole remain suitable candidates for inclusion in rotational programs, whereas flucythrinate and Bta NB-200 warrant careful reassessment of their use in the field. Notably, the recommended application strategies derived from these findings were communicated to the Animal and Plant Health Inspection Agency (APHIA) in 2019 and implemented immediately thereafter.

Susceptibility assays revealed clear temporal and spatial variation among FAW populations in Taiwan. The efficacies of etofenprox and spinetoram remained stable across regions and collection periods, supporting their continued use as reliable control options (Tables 3 and 4).

Regarding the two Bta products, the YL population (collected in 2023) exhibited different susceptibility profiles compared to the 2019 YY population: it showed lower susceptibility to Bta ABTS-1857 but higher susceptibility to Bta NB-200. No significant differences were observed between the YY and KM populations or among different laboratory generations of the YY population (Tables 5 and 6). These results indicate that even products within the same Bt subspecies can differ in field performance, underscoring the need for product- and population-specific susceptibility monitoring to guide effective Bt application strategies.

Both the YL and KM populations, collected in 2023, were directly compared in this analysis. The YL population exhibited significantly higher susceptibility to Bta NB-200 than the KM population (Table 6). Given that the recommended field concentration of Bta NB-200 is 540 μg/mL, the LC_{50} values of both populations were below this concentration. Probit back-calculation

estimated the LC_{70} values of NB-200 to be 286.71 µg/mL for the YL population and 970.47 µg/mL for the KM population (data not shown).

These results indicate that, in 2023, Bta NB-200 is expected to achieve greater than 70% mortality in the YL population (CFL < 0%), suggesting that it remains suitable for field use against this population. In contrast, its efficacy against the KM population appears insufficient, and the application of Bta NB-200 against the KM population should therefore be temporarily suspended.

Marked population- and generation-dependent differences were also observed for the chemical insecticides novaluron, flubendiamide, and chlorantraniliprole (Tables 7–9). In particular, substantially lower LC_{50} values were detected in the F_{43} progeny of YY population compared with the F_5 progeny for both diamide insecticides. Using the F_{43} progeny of YY as a susceptible reference under laboratory conditions, resistance ratios in the F_5 progeny of YY were estimated to be 9.76-fold for flubendiamide and 201.91-fold for chlorantraniliprole, indicating pronounced resistance in earlier generations.

After 40 generations of laboratory rearing of the YY population without insecticide exposure, no substantial change in susceptibility was observed for the two Bt-based insecticides, Bta NB-200 and ABTS-1857 (Tables 5 and 6). In contrast, marked reductions in resistance were detected for flubendiamide and chlorantraniliprole (Tables 8 and 9). These results suggest that resistance to diamide insecticides, particularly chlorantraniliprole, may be at least partially reversible in the absence of selection pressure. This observation is consistent with the concept that certain resistance traits impose fitness costs and may decline when selection pressure is relaxed.

From a resistance management perspective, these results highlight the potential value of temporary withdrawal or reduced use of diamide insecticides as part of an integrated resistance management strategy, provided that resistance dynamics are continuously monitored. However, it should be noted that comparisons among populations collected from different locations or years do not necessarily reflect temporal changes within a single genetic population. Without direct population genetic evidence or long-term field-based monitoring, the observed differences in susceptibility cannot be conclusively attributed to changes in field management practices or to recovery processes occurring under field conditions.

Nevertheless, the strong recovery of susceptibility observed under controlled laboratory conditions underscores the importance of minimizing sustained selection pressure and supports the strategic use of insecticide rotation to help prolong the field efficacy of diamide insecticides.

Our findings are consistent with international reports. In Nepal, chlorantraniliprole was the most effective insecticide for FAW control, followed by spinetoram and emamectin benzoate (Karki *et al.* 2023). In India, chlorantraniliprole, spinetoram, etofenprox, flubendiamide, and novaluron effectively suppressed FAW populations, with chlorantraniliprole-treated fields yielding 1.29 times that of untreated controls. (Shahi *et al*. 2024). Similarly, our results confirm that chlorantraniliprole and spinetoram remain highly effective in Taiwan. In contrast, FAW populations in Puerto Rico have evolved extreme resistance to chlorantraniliprole and flubendiamide, with resistance ratios of approximately 160- and 500-fold, respectively (Gutiérrez-Moreno *et al.* 2019).

Since the introduction of Cry1Ab-expressing maize in Brazil in 2008, continuous monitoring has shown a progressive decline in FAW susceptibility to Bt maize, accompanied by reduced field efficacy between 2010 and 2015 (Omoto *et al*. 2016). A similar trend was observed for Bta ABTS-1857 in Taiwan, where susceptibility was lower in the 2023 YL population, as indicated by a 2.8-fold higher LC_{50} compared with the 2019 YY population. In contrast, the initially invasive field population of FAW collected from mainland Taiwan in 2019 (YY) exhibited a 1.57-fold higher LC_{50} for Bta NB-200 than the

2023 YL population, although the difference was not significant (Tables 5 and 6). These differences coincide with the higher field usage of Bta ABTS-1857 relative to Bta NB-200 (as reflected by cumulative procurement volumes in Table 10); other factors, such as genetic background or historical exposure, cannot be excluded.

Similar trends have been reported in diamondback moth, in which resistant populations showed higher resistance to commercial Bt formulations than to wild-type strains (Raymond *et al*. 2013). Different Bt formulations have also been reported to vary in their efficacy against diamondback moth (Kao & Cheng 2001). These results underscore the need for careful monitoring of Bt formulation usage and rotation to maintain field efficacy and delay resistance development in FAW populations. Resistance reversal was clearly observed when FAW populations were reared without insecticide exposure. The LC_{50} values for chlorantraniliprole and flubendiamide in the YY population decreased by 201.91-fold and 9.76-fold, respectively, over 38 generations (Tables 8 and 9). Similar declines have been observed in *Plutella xylostella* (Linnaeus, 1767) and *Spodoptera exigua* (Hübner, 1808) (Pudasaini *et al*. 2022; Rabelo *et al*. 2022), reflecting fitness costs associated with resistance (Gong *et al*. 2021). These results underscore the high value of diamides in FAW control but also the urgency of implementing rotation strategies.

The YY F_5 progeny, derived from the FAW population that invaded Taiwan in 2019, exhibited higher LC_{50} values for flubendiamide compared to the YL F_2 and KM F_3 progeny collected from the field in 2023, approximately 2.71- and 4.78-fold higher, respectively (Table 8). Similarly, for chlorantraniliprole, YY F_5 showed LC_{50} values about 12.07- and 72.11-fold higher than those of the YL and KM F_6 progeny from 2023, respectively (Table 9).

The lower LC_{50} values observed in the 2023 populations relative to the initial 2019 population may partially reflect differences in susceptibility patterns influenced by coordinated FAW management in Taiwan, including centralized insecticide procurement and extension programs that encourage rotation rather than tank-mixing, although other ecological or population factors cannot be excluded.

Pesticide application history and management strategies strongly influence the frequency and distribution of resistance alleles (Yainna *et al*. 2021). Integrated pest management (IPM) programs should therefore avoid overreliance on single active ingredients and carefully manage application frequency and dosage (Paredes-Sánchez *et al*. 2021).

Maize is most vulnerable to FAW damage during the seedling stage, early whorl development, and before tasseling (Gross *et al*. 1982). Because FAW larvae feed heavily and can damage or kill the growing points, young maize plants are highly susceptible. Moreover, as larvae grow, they move deeper into the whorl, hide at the leaf base, or bore into ears, thereby reducing insecticide contact and making control

Table 10. Official procurement and field application records of selected insecticides used for the control of *Spodoptera frugiperda* in Taiwan in 2020.

Insecticide	Active ingredient	Quantity (package)	Price per package (NTD)	Application quantity (package)[z]
Spinetoram	11.7%	100 mL	750	3,282
Bta ABTS-1857	48.1%	100 g	171	44,100
Bta NB-200	54.0%	200 g	420	1,810
Novaluron	10.0%	200 mL	435	81,108
Flubendiamide	20.0%	100 g	590	58,160
Chlorantraniliprole	18.4%	80 mL	592	77,258

[z] Procurement and distribution records provided by the Animal and Plant Health Inspection Agency (APHIA), Ministry of Agriculture, Taiwan.

more difficult (Young 1979; Carvalho *et al*. 2013; Bateman *et al*. 2018). Therefore, timely insecticide application is particularly crucial. In Taiwan, maize is often grown in multiple cropping cycles per year. Insecticide applications typically begin when larvae first appear and are applied about four times per cycle (Huang 2025).

Although several insecticides, including spinetoram, etofenprox, flubendiamide, novaluron, and chlorantraniliprole, currently remain effective for FAW control in Taiwanese maize, their long-term sustainability will depend on appropriate rotation among compounds with different modes of action. Specifically, one to two insecticides with distinct modes of action should be used within each cropping cycle and rotated to alternative compounds in subsequent cycles to reduce selection pressure.

Future management programs should also integrate microbial control agents to reduce reliance on chemical insecticides and to delaythe evolution of resistance. For instance, *Metarhizium anisopliae* has demonstrated prolonged efficacy against FAW and strong potential for to suppress resistance while remaining environmentally compatible, particularly under low pest densities (Yao *et al*. 2023). Therefore, the development and field validation of microbial-based control strategies should be prioritized.

Additionally, as resistance is closely linked to detoxification enzymes and gene regulation (Carvalho *et al*. 2018; Gui *et al*. 2022; Guo *et al*. 2024), future research should focus on elucidating these molecular mechanisms. Such studies will support predictive resistance management and the development of evidence-based control strategies.

ACKNOWLEDGEMENTS

This work was supported by the Animal and Plant Health Inspection Agency, Ministry of Agriculture, Taiwan, under the projects 109 -3.1 -1(3), 110 -3.1 -1(1), 111-3.3-1(1), 112-3.3-1(1), and 113-3.3-1(1).

REFERENCES

Ahissou, B. R., W. M. Sawadogo, F. Sankara, F. C. Kambiré, A. H. Bokonon-Ganta, I. Somda, and F. J. Verheggen. 2024. Fall armyworm in West Africa: Earwig predation potential and insecticide selectivity. Biotechnol. Agron. Soc. Environ. 28:82–90. doi:10.25518/1780-4507.20731

Bateman, M. L., R. K. Day, B. Luke, S. Edgington, U. Kuhlmann, and M. J. W. Cock. 2018. Assessment of potential biopesticide options for managing fall armyworm (*Spodoptera frugiperda*) in Africa. J. Appl. Entomol. 142:805–819. doi:10.1111/jen.12565

Blanco, C. A., W. Chiaravalle, M. Dalla-Rizza, J. R. Farias, M. F. García-Degano, G. Gastaminza, ... E. Willink. 2016. Current situation of pests targeted by Bt crops in Latin America. Curr. Opin. Insect Sci. 15:131–138. doi:10.1016/j.cois.2016.04.012

Boaventura, D., A. Bolzan, F. E. O. Padovez, D. M. Okuma, C. Omoto, and R. Nauen. 2020. Detection of a ryanodine receptor target-site mutation in diamide insecticide resistant fall armyworm, *Spodoptera frugiperda*. Pest. Manag. Sci. 76:47–54. doi:10.1002/ps.5505

Carvalho, I. F., L. L. Erdmann, L. L. Machado, A. P. S. A. Rosa, M. J. Zotti, and C. G. Neitzke. 2018. Metabolic resistance in the fall armyworm: An overview. J. Agric. Sci. 10(12):426–436. doi:10.5539/jas.v10n12p426

Carvalho, R. A., C. Omoto, L. M. Field, M. S. Williamson, and C. Bass. 2013. Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. PLOS ONE 8:e62268. doi:10.1371/journal.pone.0062268

Casida, J. E. and K. A. Durkin. 2013. Neuroactive insecticides: Targets, selectivity, resistance, and secondary effects. Annu. Rev. Entomol. 58:99–117. doi:10.1146/annurev-ento-120811-153645

Chang, S. C., C. C. Lee, and M. Y. Chiang. 2026. Research and development of pathogenic microorganisms for the control of major agricultural pests. J. Taiwan Agric. Res. 75:27–49. doi:10.6156/JTAR.202603_75(1).0002

Cheng, E. Y., C. H. Kao, and C. S. Chiu. 1992. Resistance, cross-resistance and chemical control of diamondback moth in Taiwan: Recent developments. p.465–475. *in*: Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop. December 10–14, 1990. Tainan, Taiwan. Asian Vegetable Research and Development Center.

Chi, H. 2024. Probit analysis. https://zenodo.org/

records/14543846 (visit on 11/7/2024)

Cutler, G. C. and C. D. Scott-Dupree. 2007. Novaluron: Prospects and limitations in insect pest management. Pest Technol. 1:38–46.

Deshmukh, S., H. B. Pavithra, C. M. Kalleshwaraswamy, B. K. Shivanna, M. S. Maruthi, and D. Mota-Sanchez. 2020. Field efficacy of insecticides for management of invasive fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) on maize in India. Fla. Entomol. 103:221–227. doi:10.1653/024.103.0211

Finney, D. J. 1971. Probit Analysis. 3rd ed. Cambridge University Press. Cambridge, UK. 333 pp.

Goergen, G., P. L. Kumar, S. B. Sankung, A. Togola, and M. Tamò. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), a new alien invasive pest in West and Central Africa. PLOS ONE 11:e0165632. doi:10.1371/journal.pone.0165632

Gong, C., X. Yao, Q. Yang, X. Wang, Y. Zhang, Y. Wang, and L. Shen. 2021. Fitness costs of chlorantraniliprole resistance related to the *SeNPF* overexpression in the *Spodoptera exigua* (Lepidoptera: Noctuidae). Intl. J. Mol. Sci. 22:5027. doi:10.3390/ijms22095027

Gonzalez, M. R., M. Bischofberger, L. Pernot, F. G. van der Goot, and B. Frêche. 2008. Bacterial pore-forming toxins: The (w)hole story? Cell. Mol. Life Sci. 65:493–507. doi:10.1007/s00018-007-7434-y

Gross, H. R., Jr., J. R. Young, and B. R. Wiseman. 1982. Relative susceptibility of a summer-planted dent and tropical flint corn variety to whorl stage damage by the fall armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 75:1153–1156. doi:10.1093/jee/75.6.1153

Guedes, R. N. C. 2017. Insecticide resistance, control failure likelihood and the First Law of Geography. Pest Manag. Sci. 73:479–484. doi:10.1002/ps.4452

Gui, F., T. Lan, Y. Zhao, W. Guo, Y. Dong, D. Fang, ... L. Kang. 2022. Genomic and transcriptomic analysis unveils population evolution and development of pesticide resistance in fall armyworm *Spodoptera frugiperda*. Protein Cell 13:513–531. doi:10.1007/s13238-020-00795-7

Guo, Z., H. Ma, J. Tang, M. Wu, S. He, H. Wan, ... K. Ma. 2024. Chlorantraniliprole resistance in *Spodoptera frugiperda*: Resistance monitoring, resistance risk, and resistance mechanisms. J. Agric. Food Chem. 72:16651–16660. doi:10.1021/acs.jafc.4c03361

Gutiérrez-Moreno, R., D. Mota-Sanchez, C. A. Blanco, M. E. Whalon, H. Terán-Santofimio, J. C. Rodriguez-Maciel, and C. DiFonzo. 2019. Field-evolved resistance of the fall armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. J. Econ. Entomol. 112:792–802. doi:10.1093/jee/toy372

Hruska, A. J. and S. M. Gladstone. 1988. Effect of period and level of infestation of the fall armyworm, *Spodoptera frugiperda*, on irrigated maize yield. Fla. Entomol. 71:249–254. doi:10.2307/3495428

Huang, H. W. 2025. Investigation on pest management strategies for sweet corn. Res. Bull. Tainan DARES 85:67–77. (in Chinese with English abstract) doi:10.29944/BTNDAIS.202506_(85).0006

Huang, L. L., F. S. Xue, C. Chen, X. Guo, J. J. Tang, L. Zhong, and H. M. He. 2021. Effects of temperature on life-history traits of the newly invasive fall armyworm, *Spodoptera frugiperda* in Southeast China. Ecol. Evol. 11:5255–5264. doi:10.1002/ece3.7413

Kao, C. H. and E. Y. Cheng. 2001. Insecticide resistance in *Plutella xylostella* L. XI. Resistance to newly introduced insecticides in Taiwan (1990–2001). J. Agric. Res. China 50(4):80–89.

Kao, S. S. 1995. Mass-rearing of insects. Agric. Chem. Res. Inst. Pub. No. 37. Taichung, Taiwan. 8 pp. (in Chinese) doi:10.29952/YTSCTPT.199504.0001

Karki, N., A. Soti, S. Katel, R. Bhandari, N. Thapa, and S. P. S. Yadav. 2023. Field efficacy of different insecticides against fall armyworm (*Spodoptera frugiperda* J. E. Smith) in spring maize (*Zea mays* L.). AgroEnviron. Sustain. 1:93–104. doi:10.59983/s2023010202

Lazo, G. R., S. C. Chang, H. T. Shih, H. Huang, C. M. Wallis, A. Á. P. de León, and J. Chen. 2025. Mini-review on microbial pesticide research for crop protection assisted by generative AI. J. Taiwan Agric. Res. 74:399–414. doi:10.6156/JTAR.202512_74(4).0003

Lira, E. C., A. Bolzan, A. R. B. Nascimento, F. S. A. Amaral, R. H. Kanno, I. S. Kaiser, and C. Omoto. 2020. Resistance of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to spinetoram: Inheritance and cross-resistance to spinosad. Pest Manag. Sci. 76:2674–2680. doi:10.1002/ps.5812

Luginbill, P. 1928. The fall armyworm. USDA Tech. Bull. No. 34. Washington, DC. 91 pp.

Michigan State University (MSU). 2025. Arthropod Pesticide Resistance Database (APRD). https://www.pesticideresistance.org (visit on 12/7/2025).

Montezano, D. G., A. Specht, D. R. Sosa-Gómez, V. F. Roque-Specht, J. C. Sousa-Silva, S. V. Paula-Moraes, ... T. E. Hunt. 2018. Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

in the Americas. Afr. Entomol. 26:286–300. doi:10.4001/003.026.0286

Omoto, C., O. Bernardi, E. Salmeron, R. J. Sorgatto, P. M. Dourado, A. Crivellari, ... G. P. Head. 2016. Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. Pest Manag. Sci. 72:1727–1736. doi:10.1002/ps.4201

Pacheco, S., I. Gómez, M. Chiñas, J. Sánchez, M. Soberón, and A. Bravo. 2021. Whole genome sequencing analysis of *Bacillus thuringiensis* GR007 reveals multiple pesticidal protein genes. Front. Microbiol. 12:758314. doi:10.3389/fmicb.2021.758314

Paredes-Sánchez, F. A., G. Rivera, V. Bocanegra-García, H. Y. Martínez-Padrón, M. Berrones-Morales, N. Niño-García, and V. Herrera-Mayorga. 2021. Advances in control strategies against *Spodoptera frugiperda*: A review. Molecules 26:5587. doi:10.3390/molecules26185587

Pudasaini, R., M. Y. Chou, T. J. Wu, and S. M. Dai. 2022. Insecticide resistance and control failure likelihood analysis in *Plutella xylostella* (Lepidoptera: Plutellidae) populations from Taiwan. J. Econ. Entomol. 115:835–843. doi:10.1093/jee/toac048

Rabelo, M. M., I. B. Santos, and S. V. Paula-Moraes. 2022. *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) fitness and resistance stability to diamide and pyrethroid insecticides in the United States. Insects 13:365. doi:10.3390/insects13040365

Raymond, B., D. J. Wright, N. Crickmore, and M. B. Bonsall. 2013. The impact of strain diversity and mixed infections on the evolution of resistance to *Bacillus thuringiensis*. Proc. R. Soc. B 280:20131497. doi:10.1098/rspb.2013.1497

Samanta, S., M. Barman, H. Thakur, S. Chakraborty, G. Upadhyaya, D. Roy, … J. Tarafdar. 2023. Evidence of population expansion and insecticide resistance mechanism in invasive fall armyworm (*Spodoptera frugiperda*). BMC Biotechnol. 23:17. doi:10.1186/s12896-023-00786-6

Sanchis, V. and D. Bourguet. 2008. *Bacillus thuringiensis*: Applications in agriculture and insect resistance management. A review. Agron. Sustain. Dev. 28:11–20. doi:10.1051/agro:2007054

Sattelle, D. B., D. Cordova, and T. R. Cheek. 2008. Insect ryanodine receptors: Molecular targets for novel pest control chemicals. Invert. Neurosci. 8:107. doi:10.1007/s10158-008-0076-4

Shahi, S., S. Chauhan, R. Kumar, and J. K. Singh. 2024. Efficacy of newer insecticides against fall armyworm, *Spodoptera frugiperda* on maize. Uttar Pradesh J. Zool. 45(18):514–524. doi:10.56557/upjoz/2024/v45i184468

Soderlund, D. M. 2012. Molecular mechanisms of pyrethroid insecticide neurotoxicity: Recent advances. Arch. Toxicol. 86:165–181. doi:10.1007/s00204-011-0726-x

Sparks, T. C. and R. Nauen. 2015. IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Physiol. 121:122–128. doi:10.1016/j.pestbp.2014.11.014

Tomizawa, M. and J. E. Casida. 2005. Neonicotinoid insecticide toxicology: Mechanisms of selective action. Annu. Rev. Pharmacol. Toxicol. 45:247–268. doi:10.1146/annurev.pharmtox.45.120403.095930

Yainna, S., N. Nègre, P. J. Silvie, T. Brévault, W. T. Tay, K. Gordon, ... K. Nam. 2021. Geographic monitoring of insecticide resistance mutations in native and invasive populations of the fall armyworm. Insects 12:468. doi:10.3390/insects12050468

Yao, Y., Y. He, L. Zhang, K. Wang, B. Li, G. Du, ... G. Xiao. 2023. Management strategy for *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in mountain maize planting areas in China. J. Asia-Pac. Entomol. 26:102152. doi:10.1016/j.aspen.2023.102152

Young, J. R. 1979. Fall armyworm: Control with insecticides. Fla. Entomol. 62:130–133. doi:10.2307/3494089

Yu, S. J. 1991. Insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Pestic. Biochem. Physiol. 39:84–91. doi:10.1016/0048-3575(91)90216-9

臺灣秋行軍蟲 (鱗翅目：夜蛾科) 對殺蟲劑感受性變化與防治失效風險

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摘要

江明耀、張淑貞、申屠萱、蔡馨儀、張書銘、古佳崟、楊崇民。2026。臺灣秋行軍蟲 (鱗翅目：夜蛾科) 對殺蟲劑感受性變化與防治失效風險。台灣農業研究 75(2):265–280。

秋行軍蟲 (*Spodoptera frugiperda*) 於 2019 年入侵臺灣，迅速成為玉米與高粱的重要害蟲。本研究旨在評估臺灣不同地區及時間的秋行軍蟲田間族群對多種化學殺蟲劑 (包括有機磷類、除蟲菊精類、新尼古丁類、賜諾司類、二醯胺類殺蟲劑及昆蟲生長調節劑) 及 2 種蘇力菌 (*Bacillus thuringiensis*) 製劑的敏感性。在入侵初期，秋行軍蟲族群對各測試農藥之感受性差異顯著，依據推薦濃度之防治失效可能性分析 (control failure likelihood; CFL) 顯示，賜諾特、氟大滅、諾伐隆及剋安勃的防治失效風險可忽略，而蘇力菌 NB-200 與護賽寧則具有高防治失效風險。比較 2019 與 2023 年臺灣本島採集的秋行軍蟲族群，結果顯示兩年度族群之感受性模式存在差異，2023 年族群對剋安勃與諾伐隆之半數致死濃度 (LC_{50}) 顯著較低，但對蘇力菌 ABTS-1857 的之 LC_{50} 顯著較高。至於依芬寧、賜諾特及蘇力菌 NB-200 族群間感受性差異不顯著。田間族群 YY 品系在經過 38 代未接觸殺蟲劑的實驗室內飼養後，其對氟大滅與剋安勃的感受性顯著回復，LC_{50} 分別下降 9.76 倍與 201.91 倍，顯示殺蟲劑感受性隨未接受選汰壓力時間大幅回升。綜合而言，本研究結果突顯選汰壓力在秋行軍蟲抗藥性消長動態中的關鍵作用，支持以藥劑輪用及策略性暫停使用高風險藥劑為核心之綜合害蟲管理 (integrated pest management; IPM) 策略。

關鍵詞：秋行軍蟲、殺蟲劑抗藥性、二醯胺類殺蟲劑、防治失效可能性、害蟲綜合管理。

投稿日期：2025 年 10 月 16 日；接受日期：2025 年 12 月 19 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):281–291 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0008

Research Article

Development of a Liquichip-Based Assay for Detection of *Paracidovorax citrulli* in Cucurbits

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Abstract

Tsai, C. H., Y. T. Liu, S. Y. Lin, C. S. Tseng, and C. P. Kuan. 2026. Development of a Liquichip-based assay for detection of *Paracidovorax citrulli* in cucurbits. J. Taiwan Agric. Res. 75(2):281–291.

Bacterial fruit blotch (BFB), caused by *Paracidovorax citrulli*, threatens cucurbit production worldwide and often results in severe economic losses. In this study, we developed a detection assay for sensitive detection of *P. citrulli*. The assays employ polymerase chain reaction (PCR) with specific co-amplification of a plant gene as an internal control from total nucleic acids, enabling parallel detection and minimizing the risk of false-negative PCR results during routine testing and eliminating the need to remove contaminating plant DNA from extracts. The assay exhibited high specificity, as no cross-reactions were observed with non-target bacterial species or with nucleic acid extracted from healthy cucurbit tissues. Sensitivity testing demonstrated reliable detection of low concentrations of pathogen DNA, and the method was validated using artificially inoculated seedlings and field samples. Compared with conventional PCR, the multiplex detection assay provides higher throughput and quantitative readouts based on mean fluorescence intensity. This platform offers a reliable tool for seed health testing, quarantine surveillance, and integrated management of BFB in cucurbit crops.

Key words: *Paracidovorax citrulli*, Bacterial fruit blotch, Cucurbits, Diagnosis.

INTRODUCTION

Bacterial fruit blotch (BFB) is a devastating seedborne disease of cucurbits, primarily affecting *Citrullus lanatus* (watermelon) and *Cucumis melo* (melon). The causal agent was historically identified as *Acidovorax citrulli* (Schaad *et al*. 1978; Burdman & Walcott 2012), but recent phylogenomic analyses led to its reclassification as *Paracidovorax citrulli* (Du *et al*. 2023). This taxonomic update reflects an improved understanding of the *Comamonadaceae* lineage and underscores the need to update diagnostic protocols and reference databases accordingly.

BFB has caused serious epidemics worldwide, resulting in significant economic losses in watermelon and melon seed production. The bacterium is primarily transmitted through contaminated seeds, which serve as the main inoculum source for field outbreaks and facilitate international spread via trade (Burdman & Walcott 2012). Infected seedlings exhibit cotyledon necrosis, seedling wilt, and fruit lesions under

Received: November 27, 2025; Accepted: March 6, 2026.
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warm and humid conditions, while latent seed infections remain symptomless but highly infectious. Therefore, sensitive detection of *P. citrulli* is critical for effective disease management and phytosanitary certification.

In Taiwan, BFB was first reported in muskmelon and watermelon in the late 1990s, where disease incidence reached economically damaging levels (Cheng *et al*. 2000). The pathogen remains an occasional but notable threat to local cucurbit production. However, despite national surveillance and quarantine measures, the lack of a rapid, high-throughput detection platform remains a key limitation for seed-health testing programs. Diagnostic methods for *P. citrulli* have traditionally relied on grow-out assays and serological methods (Wang & Cheng 2001; Azman Husni *et al*. 2021). The enzyme-linked immunosorbent assay (ELISA) has been widely used but often lacks sensitivity for detecting low bacterial titers (Hopkins *et al*. 1996). Polymerase chain reaction (PCR) and real-time PCR offer greater sensitivity and specificity; however, their throughput remains limited in large-scale seed testing programs (Song *et al*. 1995; Schaad *et al*. 2003). The novel detection method is based on Liquichip technology, using a chemiluminescent detection and analysis system, in combination with multiplex polymerase chain reaction. This assay can simultaneously detect multiple pathogens within a single reaction tube (Lim *et al*. 2016). Although PCR-based assays are widely used for detecting *P. citrulli*, they are generally limited to single-target analysis and can be affected by inhibitory substances present in seed extracts. Liquichip technology enables high-throughput, multiplex detection of multiple targets in a single reaction, making it more suitable for large-scale seed health and quarantine screening. In addition, the flexible design of Liquichip assays allows new targets to be incorporated without extensive re-optimization. Therefore, the development of a Liquichip-based assay provides a complementary and efficient diagnostic approach for bacterial fruit blotch detection.

The aim of this study was to develop a Liquichip-based assay for detecting *P. citrulli*. The assay enables simultaneous detection of the target pathogen and plant internal control genes, and its specificity and sensitivity were systematically evaluated. The performance of the Liquichip assay was further compared with that of conventional PCR methods.

MATERIALS AND METHODS

Plant and bacterial strains

Seedlings of watermelon (*Ci. lanatus* (Thunb.) Matsum. & Nakai) and melon (*Cu. melo* L.) at the two- to three-leaf stage were used for all experiments. Seeds were grown in a controlled-environment growth chamber maintained at 25 ± 2℃ with a 16 h light/8 h dark photoperiod and approximately 70% relative humidity. Plants were watered daily and fertilized weekly with a balanced nutrient solution until used for inoculation or sampling. The bacterial pathogen, *P. citrulli* (syn. *A. avenae* subsp. *citrulli*) AAC33 (strain AAC33) was isolated from infected watermelon tissues and maintained at the Taiwan Agricultural Research Institute (TARI), Taichung, Taiwan. For experimental use, the bacterial culture was grown on nutrient agar (NA) plates at 28℃ for 48 h and stored at 4℃ for short-term preservation. Liquid cultures were prepared in nutrient broth (NB) and incubated at 28℃ with shaking at 200 rpm overnight. Reference bacterial strains used for specific testing included *Xanthomonas axonopodis* pv. *vesicatoria* (XV43), *Pseudomonas* spp. (P3), *Erwinia carotovora* (BCRC13147), *Bacillus subtilis* (BCRC6633), and *Escherichia coli* (DH5α). These strains were obtained from the TARI culture collection. All bacterial isolates were routinely cultured on NA or NB at 28℃ and stored at -80℃ in 20% glycerol for long-term preservation.

Total DNA extraction

Genomic DNA of *P. citrulli* was extracted

from pure bacterial cultures using a commercial bacterial DNA extraction kit according to the manufacturer's instructions. Briefly, a single bacterial colony was cultured in nutrient broth at 28℃ for 24 h with shaking, then centrifugated to collect the cells. Genomic DNA was purified, eluted in nuclease-free water, and stored at -20℃ until use. DNA concentration and purity were measured using a spectrophotometer, and the extracted DNA was used as the template for subsequent PCR-based assays.

Primer design

Specific primers targeting *P. citrulli* (AAC) were designed for the ribosomal gene using PrimerPlex 2.0 (PREMIER Biosoft, Palo Alto, CA, USA), based on available nucleotide sequences of the pathogen and host plant genomes. Candidate primers were verified for specificity using Basic Local Alignment Search Tool (BLAST) searches against the National Center for Biotechnology Information (NCBI) database to avoid cross-reactivity with other cucurbit pathogens. PrimerPlex 2 (PRIMER Biosoft, Palo Alto, CA, USA) was used to design target-specific primer extension/allele-specific primer extension (TSPE/ASPE) primers for multiplex PCR reactions. Final primer sequences used in this study are listed in Table 1.

Specificity of PCR for *P. citrulli*

Each 20 μL reaction contained 1 U Taq DNA polymerase (Platinum II, Invitrogen, Carlsbad, CA, USA), 2 μL of 10x PCR buffer, 400 μM dNTPs, 0.4 μM of each primer, 5–10 ng of template cDNA, and ddH_2O to 20 μL and PCR amplification was performed with thermal cycling consisted of 94℃ for 5 min, followed by 35 cycles of 94℃ for 30 s, 55℃ for 30 s, and 68℃ for 2 min, with a final extension at 68℃ for 10 min. PCR products were resolved on 2% agarose gels, stained with ethidium bromide, and imaged under UV light.

TSPE/ASPE

The probes and primers used in this study are listed in Table 1. Each probe has two functional regions: the 3′ end (18–30 nts) acts as a primer in the TSPE reaction, complementary to the target gene, while the 5′ end contains a Target Amplification gene (TAG) sequence for xTAG bead hybridization. ASPE and hybridization to xTAG microspheres were performed in a 20 μL reaction containing 5 μL of PCR product, 1× TSPE buffer, 1.0 mM $MgCl_2$, 0.05 mM of each TAG-ASPE primer, 0.75 U Tsp DNA polymerase, 5 mM of each dNTP except dCTP, and 5 mM biotin-14-dCTP (Invitrogen, Carlsbad, CA,

Table 1. Primers used in this study.

Pathogen/gene target	Target region[z]	Sequence (5′-3′)[y]	Position[x]	Amplicon size	MagPlex-TAG bead address[w]
Paracidovorax citrulli	F1	CGACATATGCAACGACTTA	2066341-2066579	239 bp	53
	R1	TGACGGTGGAATCAAATC			
	T	TTAACAACTTATACAAACACAAACTGCCG AGATAGCCATTACCAGAC -3			
Cyoxid	F	GAGGCAACAACCAGAATGAAAT	75101-75403	303 bp	26
	R	TCTCCACTAACCACAAGGATATA			
	T	TACATTCAACACTCTTAAATCAAAGCCCAT CACTCCAGCAATG			

[z] F, R, and T represent forward primer, reverse primer, and Target Amplification gene-allele-specific primer extension (TAG-ASPE) primer, respectively.
[y] Underlined indicates the MagPlex-TAG sequences provided by Luminex Corporation.
[x] Position of primers for *P. citrulli* and Cyoxid are based on the accession number CP000512.1 and MF034193, respectively.
[w] The MagPlex-bead product number assigned by Luminex Corporation.

USA). TSPE thermocycling conditions were: 94℃ for 2 min, then 30 cycles of 94℃ for 30 s, 55℃ for 30 s, and 74℃ for 1 min.

PCR products were verified by 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light.

Liquichip analysis

Detection of TSPE products with biotin was carried out using flow cytometry and magnetic bead hybridization. All xTAG magnetic bead sets were obtained from Luminex (Austin, TX, USA). Each bead set carried complementary anti-TAG sequences paired with specific TSPE probes, enabling allelic-specific hybridization.

Hybridization reactions were performed in 96-well plates, each with a total volume of 50 μL. This included 25 μL of the xTAG bead mixture (100 beads μL^{-1} in 2× tetramethylammonium chloride (TMAC) buffer: 0.4 M NaCl, 0.2 M Tris, 16% Triton X-100, pH 8.0), 5 ul TSPE extension product, and each MagPlex-TAG bead set (Table 1). The final volume was adjusted with distilled water.

The hybridization procedure: 25 μL of bead mix, 5 μL of ASPE reaction product, and 20 μL of water were incubated at 37℃ for 30 min. No-template controls were included as background references.

After hybridization, the beads were collected with a magnetic separator (Luminex, Austin, TX, USA) and washed twice with 75 μL of 1× TMAC buffer. Beads were then resuspended in 75 μL of 1× TMAC buffer containing 4 μg mL^{-1} streptavidin-R-phycoerythrin (Invitrogen, Carlsbad, CA, USA) and incubated at 37℃ for 15 min.

The final products were analyzed on the Liquichip 200 workstation at 37℃. Results were expressed as mean fluorescence intensity (MFI). For each bead set, a signal three times higher than the highest background MFI was considered positive.

Hybridization and fluorescence detection

For hybridization, 5 μL of PCR product was combined with 2,500 coupled beads in 1.5× TMAC buffer (final volume 50 μL). Samples were denatured at 95℃ for 5 min, then hybridized at 50℃ for 20 min. Beads were incubated with streptavidin-R-phycoerythrin (Molecular Probes, Eugene, OR, USA) at 50℃ for 15 min. Fluorescence was measured on the Liquichip 200 system (Qiagen, Hilden, Germany), and MFI values were recorded.

Specificity and sensitivity assay

The specificity of the assay was evaluated using total DNA extracted from *P. citrulli* isolates, unrelated bacterial pathogens (*X. axonopodis* pv. *vesicatoria*, *Pseudomonas* spp., *E. carotovora*, *B. subtilis*, and *E. coli*, as well as healthy plant tissues. Each reaction was performed in triplicate and independently repeated.

To assess detection sensitivity, genomic DNA from *P. citrulli*-infected watermelon plants was used as a template. Ten-fold serial dilutions ranging from 10ng to 10^{-6} ng were prepared in diethyl pyrocarbonate (DEPC)-treated water. Each dilution was tested in parallel by conventional PCR and the Liquichip assay, with all reactions performed in triplicate. For Liquichip analysis, results were recorded as MFI values, and a reaction was considered positive when the MFI exceeded at least three times the background signal.

Inoculation and disease assessment

P. citrulli was cultured in NB medium, incubated overnight at 28℃, and adjusted to approximately 10^9 CFU mL^{-1}. Seedlings were inoculated by the needle-wound inoculation method, in which bacterial suspensions were applied to wounded leaf surfaces created with a sterile needle. Sterile distilled water was used as the negative control. Disease symptoms were recorded daily for 10 d post-inoculation, and symptomatic leaf tissues were collected for subsequent analyses. Leaf samples (~0.1 g) were collected from watermelon plants grown in fields in Taiwan. Total DNA extraction and PCR were performed as described above. Detection was performed using the Liquichip assay, and results were validated by conventional PCR.

RESULTS

Evaluation of analytical cross-reactivity in the Liquichip assay for AAC detection

To assess assay specificity, DNA extracts from melon and watermelon tissues were subjected to PCR with *P. citrulli*-specific primers, yielding a 239-bp amplicon. A plant cyoxid gene fragment (303 bp) was co-amplified as an internal control to verify DNA quality. The amplified products were hybridized with microsphere-conjugated probes and analyzed using the Liquichip system. Strong fluorescence signals were detected only in samples infected with *P. citrulli*, whereas no cross-reactivity was observed in healthy cucurbit tissues or negative controls (sterile water). The cyoxid internal control was consistently detected in all reactions, confirming successful DNA extraction and amplification (Fig. 1).

Specificity testing further demonstrated that the Liquichip assay reacted exclusively with *P. citrulli* and showed no signal with other bacterial species, including *E. coli*, *B. subtilis*, *Pseudomonas* spp., *X. axonopodis* pv. *vesicatoria*., or *E. carotovora* (Fig. 2).

Detection limit analysis of Liquichip and RT-PCR

To determine the detection limit, a 10-fold serial dilution of total DNA extracted from *P. citrulli*-infected watermelon was prepared and used as the template for both PCR and Liquichip assays. PCR amplification followed by agarose gel electrophoresis revealed that *P. citrulli* could be specifically detected up to a dilution of 10^{-5}, corresponding to approximately 10^{-4} ng of DNA. Similarly, the Liquichip system also produced detectable signals at the 10^{-5} dilution, corresponding to about 10^{-4} ng of target DNA. These results indicate that the sensitivity of the Liquichip assay is comparable to that of PCR assay (Figs. 3 and 4).

Field evaluation of AAC detection using the Liquichip assay

Disease symptoms developed progressively in both melon and watermelon seedlings after inoculation with *P. citrulli* (Table 2, Fig. 5). In melon, visible symptoms appeared by dpi (day of post infection) 2 in some plants and reached 100% incidence by dpi 7. Watermelon showed

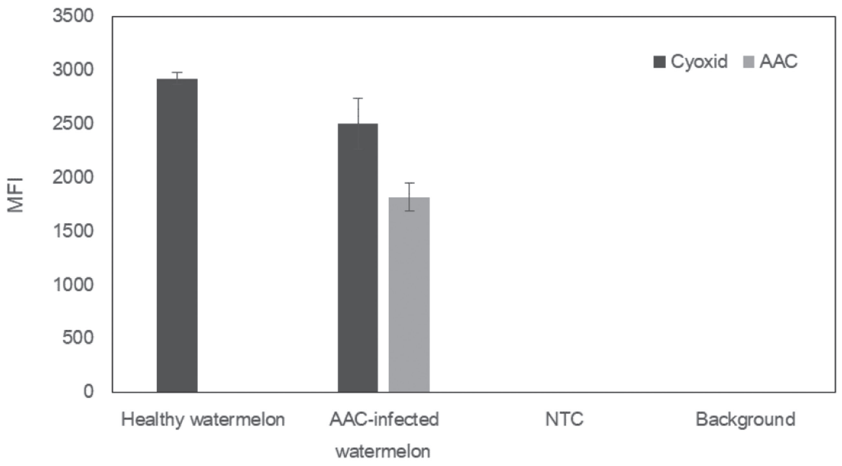

Fig. 1. Specificity assay of Liquichip for the detection of *Paracidovorax citrulli*-infected plant. Tested samples included *P. citrulli*-infected watermelon and healthy watermelon. All reactions were performed with dual probes simultaneously, and background signal values were subtracted. Each bar represents the average MFI of the Liquichip assay. NTC indicates no-template reaction, respectively. Error bars represent the standard deviations from three replicate reactions. MFI: mean fluorescence intensity. *Acidovorax avenae* subsp. *citrulli* (AAC)-infected watermelon: *P. citrulli*-infected watermelon.

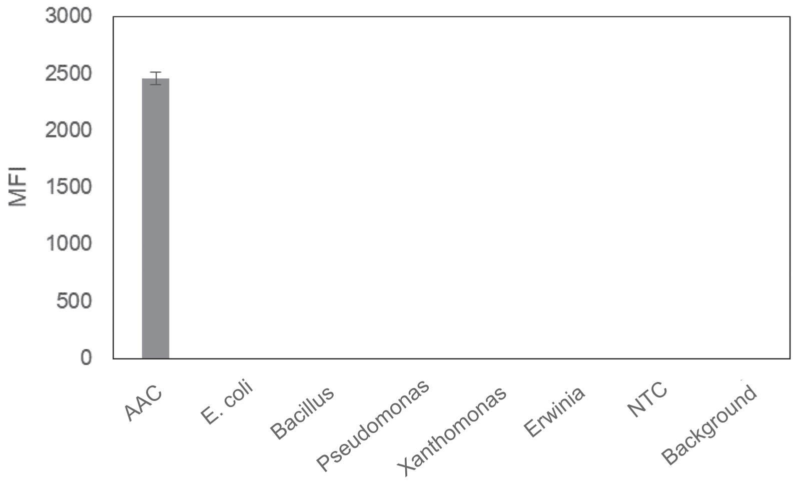

Fig. 2. Specificity assay of Liquichip for detection of *Paracidovorax citrulli*. Tested samples included *P. citrulli* (*Acidovorax avenae* subsp. *citrulli*; AAC), *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas* spp., *Xanthomonas axonopodis* pv. *vesicatoria*, *Erwinia carotovora*, and healthy watermelon. All reactions were performed with dual probes simultaneously, and background signal values were subtracted. Each bar represents the average MFI of the Liquichip assay. NTC indicates no-template reaction, respectively. Error bars represent the standard deviations from three replicate reactions. MFI: mean fluorescence intensity.

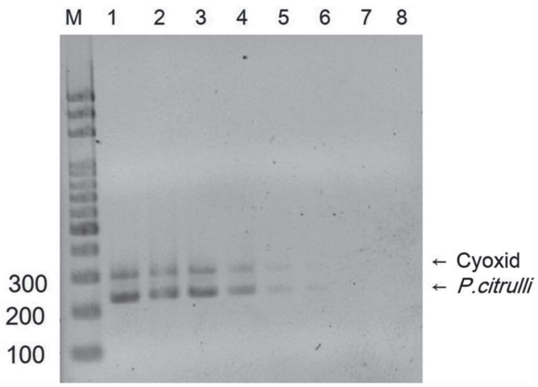

Fig. 3. Sensitivity evaluation of polymerase chain reaction (PCR) for the detection of *Paracidovorax citrulli*. PCR products amplified from a 10-fold serially diluted *P. citrulli*-infected watermelon. Lane M represents the 100-bp ladder. Lane 1–8: templates of total DNA were from 10 ng to 10^{-6} ng serial dilutions, respectively.

a similar disease progression but with slightly faster detection using the Liquichip assay. At dpi 2, Liquichip detected *P. citrulli* in all three watermelon plants (100%), while only two of three melon plants (67%) tested positive. By dpi 4, both hosts reached full detection with Liquichip, whereas PCR required until dpi 7 for complete detection in both species.

DISCUSSION

Bacterial fruit blotch remains a major limiting factor in cucurbit production (Burdman & Walcott 2012). The primary strategy for disease management is early and accurate detection, as no effective resistant cultivars are available. Traditional assays, such as isolation and ELISA, provide useful diagnostic tools but are often constrained by sensitivity (Hopkins *et al*. 1996; Schaad *et al*. 2003). PCR assays have improved sensitivity and specificity (Song *et al*. 1995; Walcott & Gitaitis 2000), but their use for routine large-scale testing remains limited. The International Seed Testing Association (ISTA) currently recommends ELISA and PCR-based methods for the detection of *P. citrulli* in seed health testing (ISTA 2022). While ELISA is widely adopted because of its simplicity and ability to screen many samples simultaneously, its sensitivity is insufficient when pathogen titers are low (Hopkins *et al*. 1996; Burdman & Walcott 2012). PCR improves detection sensitivity but

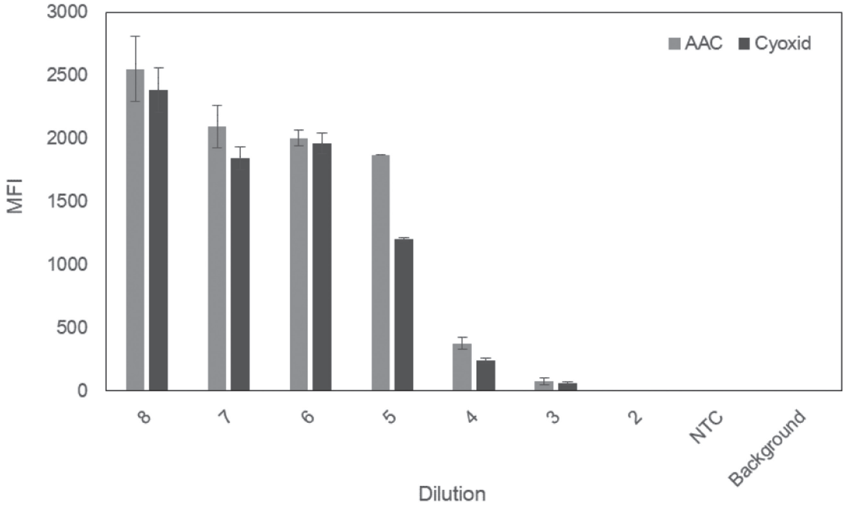

Fig. 4. Sensitivity evaluation of Liquichip for the detection of *Paracidovorax citrulli*. DNA extracted from *P. citrulli*-infected watermelon was serially diluted from 10 ng to 10^{-6} ng. Each bar represents the average MFI of the LiquiChip assay. NTC indicates no-template reaction, respectively. Error bars represent the standard deviations from three replicate reactions. MFI: mean fluorescence intensity. *Acidovorax avenae* subsp. *citrulli* (AAC)-infected watermelon: *P. citrulli* infected watermelon.

Table 2. Disease development and detection of *Paracidovorax citrulli* in melon and watermelon seedlings.

Host	dpi[z]	Symptom observation	PCR[y] positives (n/3, %)	Liquichip positives (n/3, %)
Melon	0	No visible symptom	0/3 (0%)	0/3 (0%)
Melon	2	Small water-soaked lesions	1/3 (33%)	2/3 (67%)
Melon	4	Necrotic spots with chlorotic	2/3 (67%)	3/3 (100%)
Melon	7	Expanded blotches	3/3 (100%)	3/3 (100%)
Melon	10	Expanded blotches	3/3 (100%)	3/3 (100%)
Watermelon	0	No visible symptom	0/3 (0%)	0/3 (0%)
Watermelon	2	Small water-soaked lesions	2/3 (33%)	3/3 (100%)
Watermelon	4	Necrotic spots with chlorotic	2/3 (67%)	3/3 (100%)
Watermelon	7	Expanded blotches	3/3 (100%)	3/3 (100%)
Watermelon	10	Expanded blotches	3/3 (100%)	3/3 (100%)

[z] dpi: day of post infection; n/3, %: percentage of positives = (n/3) × 100%.
[y] PCR: polymerase chain reaction.

requires labor-intensive nucleic acid extraction and electrophoretic analysis of products, which limits its applicability in high-throughput seed testing laboratories (Minsavage *et al*. 1990). The Liquichip assay developed here demonstrated high specificity and sensitivity comparable to PCR. In our evaluation, the Liquichip assay achieved detection sensitivity down to 10^{-4} ng of *P. citrulli* DNA, likewise, PCR reliably detected 10^{-4} ng as well. This level of sensitivity surpasses the detection capacity of ELISA and is comparable to, or better than, PCR-based assays (Bergervoet *et al*. 2008). The specificity assessment confirmed that the assay was highly selective for *P. citrulli*, with no cross-reaction observed with unrelated cucurbit pathogens or healthy plant extracts. Importantly, the inclusion of an internal control (plant gene) ensured DNA extraction quality,

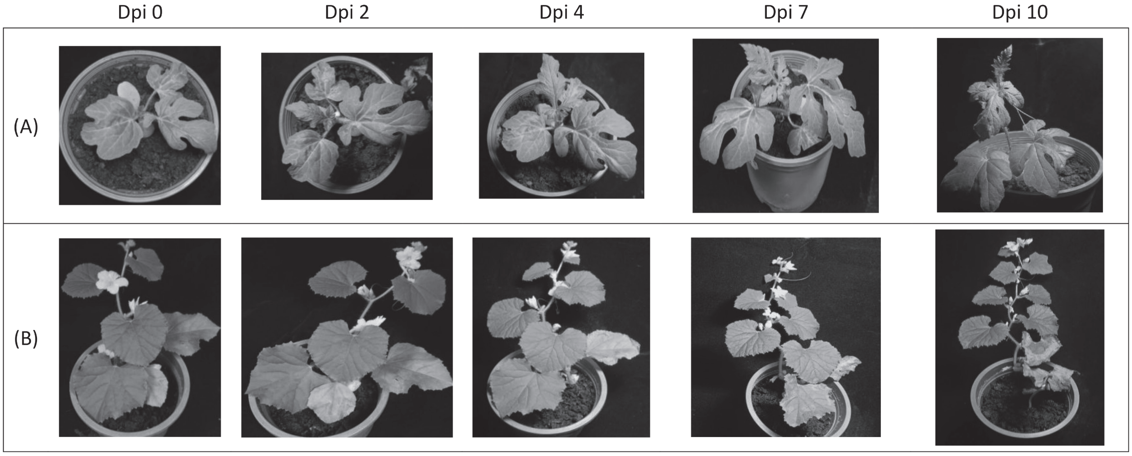

Fig. 5. Disease progression and sampling timeline of watermelon and melon plants following inoculation with *Paracidovorax citrulli* (*Acidovorax avenae* subsp. *citrulli*; AAC). Plants were monitored daily for symptom development from 1 to 10 d post-inoculation (dpi). Leaf samples were collected at 0, 2, 4, 7, and 10 dpi for subsequent pathogen detection and analysis. (A) watermelon; (B) melon.

thereby reducing the risk of false negatives, a key consideration in seed certification programs. Beyond sensitivity, the multiplexing capacity of the Liquichip system allows simultaneous detection of multiple pathogens in a single reaction. This greatly enhances testing efficiency, reducing reagent use and labor compared with conventional PCR or ELISA (Bergervoet *et al*. 2008). Although seed testing is not currently used in seed health laboratories, where a large number of samples must be screened, the semi-automated nature of the Liquichip platform further reduces dependence on specialized personnel and enables reliable, high-throughput testing.

Our results indicate that the differing symptom severity between watermelon and melon may be influenced by variations in pathogen concentration, which could reflect host-specific responses to BFB infection. Watermelon and melon are generally recognized as highly susceptible hosts, typically supporting rapid bacterial multiplication and producing typical BFB symptoms, whereas cucumber and squash often exhibit low-titer or latent infections with mild or absent symptoms (Hopkins *et al*. 1996; Burdman & Walcott 2012). These host-associated differences directly influence diagnostic assay performance because pathogen loads in different cucurbits can vary by several orders of magnitude. Quantitative analyses have shown that pathogen titers in symptomatic watermelon seedlings frequently exceed 10^6 CFU g^{-1}, while cucumber tissues often harbor only 10^2–10^3 CFU g^{-1}, levels that pose substantial detection challenges for ELISA and, in some cases, even for standard PCR assays (Walcott & Gitaitis 2000; Schaad *et al*. 2003). These limitations have resulted in false negatives in seed health certification programs, particularly when dealing with asymptomatic low-level infections (Song *et al*. 1995; Hopkins *et al*. 1996). Although earlier PCR-based assays provided higher sensitivity than serological methods, their analytical performance remained partially dependent on pathogen concentration and host species.

In Taiwan, BFB has been recognized as a persistent concern for both melon and watermelon production. Outbreaks were first documented in the 1990s, and periodic re-emergence continues to constrain seedling production and commercial fruit yields (Cheng *et al*. 2000). The pathogen has been detected in domestic and imported seed lots, underscoring the importance of rapid and highly sensitive diagnostic systems for

quarantine screening. Recent surveys in Taiwan have demonstrated considerable variability in bacterial loads among cucurbit hosts, consistent with global findings (Cheng *et al*. 2000). These host-dependent variations further underscore the necessity for diagnostic platforms that can accurately detect *P. citrulli* across a wide range of cucurbits, regardless of symptom expression or bacterial concentration.

The Liquichip assay developed in the present study offers clear advantages over conventional molecular and serological methods. By integrating multiplex PCR with bead-based hybridization and chemiluminescent detection, the diagnostic platform enhances signal-to-noise ratios and improves analytical sensitivity (Lim *et al*. 2016), particularly for low-titer infections or testing of asymptomatic cucurbits or seedling materials. This capacity for high-throughput detection is especially valuable for large-scale seed testing programs, where early detection of asymptomatic contamination is critical. Overall, combining molecular specificity with bead-based multiplex detection provides a powerful tool to strengthen BFB surveillance, support seed certification, and reduce the risk of pathogen dissemination.

In conclusion, the Liquichip assay demonstrated performance comparable to PCR for the detection of *P. citrulli*, providing sensitivity and greater diagnostic efficiency. Its advantages, including sensitivity and reduced labor requirements, make it a valuable alternative to conventional seed health assays. By enabling rapid and reliable detection of BFB, this technology has the potential to enhance disease diagnosis and contribute to the sustainable production of cucurbit crops.

REFERENCES

Azman Husni, A. A., S. I. Ismail, N. Jaafar, and D. Zulperi. 2021. Etiology, diagnostic approaches and management strategies of *Acidovorax citrulli*, a bacterial fruit blotch pathogen of cucurbits. Plant Protect. Sci. 57:75–94. doi:10.17221/52/2020-PPS

Bergervoet, J. H. W., J. Peters, J. R. C. M. van Beckhoven, G. W. van den Bovenkamp, J. W. Jacobson, and J. M. van der Wolf. 2008. Multiplex microsphere immuno-detection of potato virus Y, X and PLRV. J. Virol. Methods 149:63–68. doi:10.1016/j.jviromet.2008.01.020

Burdman, S. and R. Walcott. 2012. *Acidovorax citrulli*: Generating basic and applied knowledge to tackle a global threat to the cucurbit industry. Mol. Plant Pathol. 13:805–815. doi:10.1111/j.1364-3703.2012.00810.x

Cheng, A. H., Y. L. Hsu, T. C. Huang, and H. L. Wang. 2000. Susceptibility of cucurbits to *Acidovorax Avenae* subsp. *citrulli* and control of fruit bloth on melon. Plant Pathol. Bull. 9:151–156. (in Chinese with English abstract) doi:10.6649/PPB.200012_9(4).0004

Du, J., Y. Liu, and H. Zhu. 2023. Genome-based analyses of the genus *Acidovorax*: Proposal of the two novel genera *Paracidovorax* gen. nov., *Paenacidovorax* gen. nov. and the reclassification of *Acidovorax* antarcticus as *Comamonas antarctica* comb. nov. and emended description of the genus *Acidovorax*. Arch. Microbiol. 205:42. doi:10.1007/s00203-022-03379-7

Hopkins, D. L., J. D. Cucuzza, and J. C. Watterson. 1996. Wet seed treatments for the control of bacterial fruit blotch of watermelon. Plant Dis. 80:529–532. doi:10.1094/PD-80-0529

International Seed Testing Association (ISTA). 2022. International Rules for Seed Testing 2022. ISTA. Bassersdorf, Switzerland. 300 pp.

Lim, M. S., S. M. Kim, and S. H. Choi. 2016. Simultaneous detection of three lily-infecting viruses using a multiplex Luminex bead array. J. Virol. Methods 231:34–37. doi:10.1016/j.jviromet.2016.02.007

Minsavage, G. V., D. Dahlbeck, M. C. Whalen, B. Kearny, U. Bonas, B. J. Staskawicz, and R. E. Stall. 1990. Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. *vesicatoria*-pepper interactions. Mol. Plant Microbe Interact. 3:41–47. doi:10.1094/MPMI-3-041

Schaad, N. W., R. D. Frederick, J. Shaw, W. L. Schneider, R. Hickson, M. D. Petrillo, and D. G. Luster. 2003. Advances in molecular-based diagnostics in meeting crop biosecurity and phytosanitary issues. Annu. Rev. Phytopathol. 41:305–324. doi:10.1146/annurev.phyto.41.052002.095435

Schaad, N. W., G. Sowell Jr., R. W. Goth, R. R. Colwell, and R. E. Webb. 1978. *Pseudomonas pseudoalcaligenes* subsp. *citrulli* subsp. nov. Intl. J. Syst. Bacteriol. 28:117–125. doi:10.1099/00207713-28-1-117

Song, W. Y., G. L. Wang, L. L. Chen, H. S. Kim, L. Y. Pi, T. Holsten, … P. Ronald. 1995. A receptor kinase-

like protein encoded by the rice disease resistance gene, Xa21. Science 270:1804–1806. doi:10.1126/science.270.5243.1804

Walcott, R. R. and R. D. Gitaitis. 2000. Detection of *Acidovorax avenae* subsp. *citrulli* in watermelon seed using immunomagnetic separation and the polymerase chain reaction. Plant Dis. 84:470–474. doi:10.1094/PDIS.2000.84.4.470

Wang, H. L. and A. H. Cheng. 2001. Development of serological detection technique for bacterial fruit blotch *Acidovorax avenae* subsp. *citrulli* in cucurbit crops. Plant Pathol. Bull. 10:129–138. (in Chinese with English abstract) doi:10.6649/PPB.200109_10(3).0005

建立以 Liquichip 為基礎之葫蘆科作物中 *Paracidovorax citrulli* 檢測分析方法

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摘要

蔡佳欣、劉雅婷、林思妤、曾清山、關政平。2026。建立以 Liquichip 為基礎之葫蘆科作物中 *Paracidovorax citrulli* 檢測分析方法。台灣農業研究 75(2):281–291。

由 *Paracidovorax citrulli* 引起的細菌性果斑病 (bacterial fruit blotch; BFB) 對全球瓜類作物生產造成嚴重威脅，並經常導致顯著的經濟損失。本研究建立一套可高靈敏偵測 *P. citrulli* 的檢測方法。該檢測系統採用聚合酶連鎖反應 (polymerase chain reaction; PCR) 並同時共擴增植物基因作為內部控制，以全核酸進行分析偵測，有效降低例行檢測中產生偽陰性結果的風險，並降低樣品中植物 DNA 污染。結果顯示，本方法具有高度專一性，未與非目標細菌菌種或健康瓜類組織所萃取之 DNA 產生交叉反應。靈敏度測試證實本系統可穩定偵測低濃度之病原菌 DNA，且已成功應用於人工接種幼苗感染田間樣品之驗證。與傳統 PCR 方法相比，本檢測系統具備較高的檢測通量，並可依螢光強度提供定量化的分析結果。本平臺可作為瓜類果斑病於種子健康檢測、檢疫監測及整合性病害管理之可靠診斷工具。

關鍵詞：細菌性果斑病、瓜類、分子診斷、西瓜。

投稿日期：2025 年 11 月 27 日；接受日期：2026 年 3 月 6 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):293–306 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0009

Research Article

Identification of *Pseudomonas corrugata*, the Causal Agent of Tomato Pith Necrosis, and Screening of Agrochemicals

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Abstract

Tsai, C. H., S. L. Hwang, M. J. Lin, C. Y. Ku, and T. H. Hung. 2026. Identification of *Pseudomonas corrugata*, the causal agent of tomato pith necrosis, and screening of agrochemicals. J. Taiwan Agric. Res. 75(2):293–306.

In 2014, tomato plants grown in a field located in the Waipu District, Taichung City, Taiwan, exhibited stunted growth and increased fruit abscission. Affected plants exhibited epidermal browning on the stems, and longitudinal sections of the stem revealed browning of the vascular bundles. Microscopic examination of the symptomatic stem tissues revealed abundant bacterial presence, indicating a bacterial infection. On nutrient agar, colonies of *Pectobacterium carotovorum* subsp. *carotovorum*, the known causal agent of bacterial soft rot, was isolated from symptomatic stem tissues. In addition, the unknown yellowish bacteria were also isolated. The yellowish bacteria were identified as *Pseudomonas corrugata* based on the Biolog identification system, physiological and biochemical characterizations, polymerase chain reaction (PCR) amplification using species-specific primer sets, and bacterial gene sequence analysis. Pathogenicity tests were conducted by inoculating the bacterial isolates into healthy tomato plants, which subsequently developed vascular bundle browning extending longitudinally along the stem, resembling the symptoms observed in the field. In cases of severe infection, pith necrosis was also observed. The same bacteria were successfully re-isolated from the symptomatic tissues, thereby fulfilling Koch's postulates. This is the first report of *P. corrugata* infection on tomato in Taiwan. To manage the disease, an *in vitro* screening of commercial agrochemicals was conducted. Streptomycin, streptomycin + tetracycline, and thiophanate methyl + streptomycin produced distinct inhibitory zones against bacterial growth. Afterwards, no further tomatoes were planted in the field, and the disease did not recur.

Key words: Tomato, Pith necrosis, *Pseudomonas corrugate*, Agrochemical screening.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is an important horticultural crop in Taiwan, serving both as a fruit and a vegetable. It is widely consumed in fresh, cooked, or processed forms. The total tomato cultivation area in Taiwan in 2024 reached 3,637 ha, yielding an overall production of 27,298 Mg. The primary production regions are located in central and southern Taiwan. Chiayi County has the largest cultivation area, followed by Nantou County, according to the Agricultural Statistical Yearbook 2024 (https://agrstat.moa.gov.tw/sdweb/public/book/Book.aspx).

Received: September 11, 2025; Accepted: January 28, 2026.
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In 2014, some tomato plants showing stunted growth, premature fruit drop, and wilting were observed in a tomato flied located in the Waipu District, Taichung City (Fig. 1A). The epidermal surface of the stem exhibited brown-black patches (Fig. 1B), and longitudinal sections of symptomatic stems revealed vascular discoloration, characterized by browning and spread along the vascular bundles (Fig. 1C). Microscopic examination of the excised vascular tissues revealed abundant bacterial exudation, indicating a bacterial infection. A review of international literature indicates that various bacterial pathogens are capable of infecting vascular bundles in tomato such as *Ralstonia solanacearum* (causing bacterial wilt) (Phiri *et al*. 2024), *Pectobacterium carotovorum* (bacterial stem rot) (Caruso *et al*. 2016), *Clavibacter michiganensis* (bacterial canker) (Sen *et al*. 2015) and several bacterial species belonging to *Pseudomonas* genus including *Ps. viridiflava* (Aysan *et al*. 2004), *Ps. cichorii* (Ruan *et al*. 2019), *Ps. mediterranea* (Alippi & López 2010), *Ps. marginalis* (Bella & Catara 2010), *Ps. corrugata* (Moura *et al*. 2005; Molan & Ibrahim 2007), and *Ps. fluorescens* (Saygili *et al*. 2004; Molan & Ibrahim 2007), which are implicated in bacterial pith necrosis.

The field covered approximately 0.2 ha, with wilting symptoms observed in about 20% of the plants, resulting in significant economic losses. Therefore, this study aimed to identify the causal agents responsible for the wilt symptoms in tomato plants and to conduct *in vitro* screening of commercial agrochemicals. The results provide a reference for developing effective disease management strategies in tomato cultivation.

MATERIALS AND METHODS

The source of bacterial isolates

Samples of tomato plants exhibiting wilt and stem browning symptoms were randomly collected from a diseased field in the Waipu District, Taichung City. Infected stem tissues were excised and surface-sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 30 s,

Fig. 1. Symptoms of diseased tomato plants in the field. Infected plants showed (A) wilting of lateral branches; (B) brown-black patches on the stem epidermis; and (C) vascular bundles browning.

followed by 3 rinses with sterile water. The symptomatic tissues were then finely chopped and placed in centrifuge tubes containing sterile water, shaken to release bacteria. A loop was used to collect the bacterial suspension, which was streaked onto nutrient agar (Difco Laboratories; Becton, Dickinson and Company, Le Pont-de-Claix, France) plates. The plates were incubated at 28℃ for 2–3 d until bacterial colonies appeared. A single bacterial colony was selected and streaked onto a new nutrient agar plate, and this purification process was repeated 3 times to obtain pure bacterial isolates for further experiments.

Biolog identification system test

To simultaneously analyze different types of bacterial isolates, the Biolog identification system (Biolog Inc., Hayward, CA, USA) was used to analyze 3 bacterial isolates of yellowish colonies, numbered TW01, TW02, and TW03, and 3 bacterial isolates of white colonies, numbered TW04, TW05, and TW06. First, the isolates were cultured on a 5.7% BUG™ agar (Biolog Universal Growth Agar, Biolog Inc., Hayward, CA, USA). After culturing at 30℃ for 16–24 h in an incubator, use a cotton swab to pick up bacterial colonies and inoculate them into IF-A inoculum (Biolog Inc., Hayward, CA, USA). The concentration of the inoculum was adjusted to 90–98% T (turbidity). The prepared inoculum was then added to the Biolog GENIII reaction plate (Biolog Inc., Hayward, CA, USA), and 100 μL was added to each well. The reaction plates were incubated for 25–35 h and measured with a spectrometer. The obtained data were analyzed using the Biolog MicroLog™ 3 ver. 5.2.2 system and Biolog GENIII database (version 2.6.1) to identify bacterial species.

Physiological and biochemical analysis

Physiological and biochemical characteristics of bacterial isolates TW01, TW02, and TW03 were determined following the methods of Schaad *et al.* (2001) and Catara *et al.* (2002), with all assays conducted in duplicate. Tests performed included Gram reaction, fluorescence pigment production on King's B medium, oxidative/fermentative utilization of glucose (O/F test), levan production, oxidase activity, potato soft rot assay, arginine dihydrolase activity, hypersensitive reaction on tobacco, gelatin liquefaction, and growth at 37℃. In addition, utilization of mannitol, benzoate, sorbitol, trehalose, sucrose, D-arabinose, L-rhamnose, histamine, meso-tartrate, and 2-ketogluconate was assessed. These assays were used to determine the taxonomic affiliation of the bacterial isolates tested.

16S ribosomal RNA (rRNA) gene and DNA gyrase subunit B (*gyrB*) gene sequence analysis

According to the Biolog identification system, TW01, TW02, and TW03 were identified as *Ps. corrugata*, a bacterium that has not yet been reported to infect tomatoes in Taiwan. To further identify the bacteria isolated in this study, sequence analysis of the 16S rRNA and *gyrB* genes was performed on the nucleic acids of bacterial isolates TW01, TW02, and TW03. Nucleic acid extraction was carried out using a slight modification of the simple extraction method described by Wang *et al.* (1993). The 3 tested bacterial isolates were first streaked onto nutrient agar plates and incubated until bacterial colonies formed. A single colony was picked using a sterile toothpick and suspended in 20 μL of sterile water. Then, 20 μL of 0.4 N NaOH was added, vortexed, and left to stand for 10 min. Next, 40 μL of 1 M Tris-HCl was added and thoroughly mixed, followed by a tenfold dilution with sterile water to serve as the polymerase chain reaction (PCR) templates. PCR amplifications were performed using the bacterial universal primer pair f8-27/r1510 for the 16S rRNA gene (Lipson & Schmidt 2004) and the primer pair UP-1/UP-2r (Yamamoto & Harayama 1995) for *gyrB* gene.

Amplified DNA products were analyzed by electrophoresis on a 1.4% agarose gel to confirm expected DNA fragment sizes. The PCR products were then cloned using the T&A™ cloning kit (Yeastern Biotech, Taipei, Taiwan). After bacterial

transformation, the bacteria carrying the cloned DNA fragments were propagated, and plasmids were extracted. The extracted plasmids were subjected to PCR and electrophoresis to verify the presence of the expected DNA fragments. The successfully transformed bacteria were subsequently sent to Tri-Biotech Inc. (Taipei, Taiwan) for DNA sequencing, and the resulting DNA sequence data were then compared against the National Center for Biotechnology Information (NCBI) gene database for identification.

Specific primer pair analysis

The bacterial isolates were initially identified as *Ps. corrugata* using the Biolog identification system. However, *Ps. corrugata* and *Ps. mediterranea* are highly similar, and *Ps. mediterranea* was previously regarded as a group within *Ps. corrugata* before being reclassified as a distinct species (Catara *et al*. 2002). To verify the taxonomic identity of the bacterial isolates, PCR assays were performed using specific primer sets. The bacterial isolates TW01, TW02, and TW03, along with the reference strains *Ps. mediterranea* (DSM 16733) and *Ps. corrugata* (DSM 7228), were subjected to PCR amplification using the primer pair PC5/1 and PC5/2 specific for *Ps. corrugata*, and PC1/1 and PC1/2 specific for *Ps. mediterranea* (Catara *et al*. 2000, 2002). The PCR conditions were slightly modified as follows: The reaction mixture had a total volume of 20 μL, consisting of 1× PCR buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTP, 1.25 pmol of each primer, 0.4 units of Taq DNA polymerase, and 2 μL of template DNA. The thermal cycling conditions were as follows: initial denaturation at 94℃ for 5 min; followed by 30 cycles of 94℃ for 1 min, 62℃ for 1 min, and 72℃ for 30 s; with a final extension at 72℃ for 5 min. The amplified DNA products were analyzed by electrophoresis on a 1.4% agarose gel to verify the presence of the expected DNA fragments.

Whole-genome DNA sequence analysis

The bacterial isolate TW01 was cultured in 5 mL of nutrient broth for 24 h. Total nucleic acids were extracted from the bacterial suspension using the nucleic acid extraction kit (EasyPure Stool Genomic DNA Kit). The extracted nucleic acids were submitted to a biotechnology company for Illumina NovaSeq paired-end sequencing. Short-read sequences were de novo assembled with Velvet assembly software (version 1.2.10) to generate a draft genome. The assembled draft genome of TW01 was subsequently used to estimate digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) values for taxonomic identification. The dDDH analysis was performed using the Genome-to-Genome Distance Calculator (GGDC) with Formula 2 (Meier-Kolthoff *et al*. 2013). ANI comparisons against the *Ps. corrugata* type strains NCPPB 2445 (GenBank accession RBOJ00000000.1) and DSM7228 (GenBank accession LHVK00000000.1) were conducted using ortholog-based average nucleotide identity (OrthoANI) as described by Lee *et al*. (2016).

Koch's postulates test

To identify the causal agent of the disease, Koch's postulates were tested using 3 bacterial isolates (TW01, TW02, and TW03) obtained from the wilted tomato plant in the Waipu District. The tomato cv. 'Hong Fan' was used as the host plant. Seedlings were grown in pots until reaching approximately 60 cm in height before inoculation. Each bacterial isolate was first streaked onto nutrient agar and incubated at 28℃ for 24 h. Bacterial cells were then harvested and suspended in sterile distilled water. The concentration of each bacterial suspension was adjusted to an optical density (OD_{600}) of 0.3 (approximately 10^8 CFU mL^{-1}), as measured using a spectrophotometer (Bausch & Lomb, Bridgewater, NJ, USA), and used as the inoculum. For inoculation, one drop of the bacterial suspension was applied to the stem surface of each plant. A sterile toothpick was then used to create a small wound at the inoculation site to facilitate bacterial entry. Each plant was inoculated at 2 separate sites, and 3 plants were used for each bacterial isolate. Control plants were mock-inoculated

with sterile water. After inoculation, plants were enclosed in transparent plastic bags to maintain high humidity for 48 h, then transferred to a plant growth chamber maintained at 28℃. Symptom development was monitored daily. Upon the appearance of disease symptoms, bacteria were re-isolated from the infected stem tissues and identified using PCR with specific primer pairs to confirm that they were identical to the original bacterial isolates, thereby fulfilling Koch's postulates.

In vitro agrochemicals susceptibility test

The bacterial isolates TW01 and TW03 were selected for antimicrobial susceptibility testing. The paper disc diffusion method, as described by Adaskaveg & Hine (1985), was used to evaluate the sensitivity of the bacterial isolates to various commercially available agrochemicals at different concentrations. A total of 11 agrochemicals, known for their effectiveness against bacterial pathogens as listed in the database of the Plant Protection Information System (https://otserv2.acri.gov.tw/PPM/), were selected for testing. The concentration range for each agrochemical was based on the recommended dilution ratio on product labels, with additional tests conducted at concentrations increased and decreased by one-half to allow for comparative analysis. Four types of agrochemicals were used: (1) antibiotics, such as streptomycin (12.5%, soluble concentrate (SL), Great Victory Chemical Industry Co. Ltd., Taipei, Taiwan), streptomycin + tetracycline (10.0%, water soluble powder (SP), A11 Taiwan Pesticides Co. Ltd., Taipei, Taiwan), kasugamycin (2.0%, wettable powder (WP), Worldwide Agrochemical Co. Ltd., Taipei, Taiwan), oxolinic acid (20.0%, WP, Sumitomo Chemical Taiwan Co. Ltd., Taipei, Taiwan), and validamycin (20%, SL, Lih-Nung Chemical Co. Ltd., Yunlin, Taiwan) (2) copper-containing agrochemicals, such as copper hydroxide (53.8%, water dispersible granules (WG), Corteva Agriscience Taiwan Co., Ltd., Taipei, Taiwan) and tribasic copper sulfate (27.12%, suspension concentrate (SC), Taiwan Nissan Chemical Industries Corp., Taipei, Taiwan); (3) zinc-manganese-containing agrochemical such as mancozeb (80.0%, WP, Worldwide Agrochemical Co. Ltd., Taipei, Taiwan); (4) mixed agrochemical, such as thiophanate methyl + streptomycin (68.8%, WP, Jui Sui Corp., Taipei, Taiwan), kasugamycin + copper oxychloride (81.3%, WP, Worldwide Agrochemical Co. Ltd., Taipei, Taiwan), fosetyl-aluminium + isotianil (77%, WG, Bayer Taiwan Co. Ltd., Taipei, Taiwan). The antimicrobial activity of the test agrochemicals was evaluated using an agar diffusion assay. Bacterial cultures were first prepared as suspensions and adjusted to a final concentration of 10^8 CFU mL^{-1}. 0.1 mL of the bacterial suspension was mixed thoroughly with 6 mL of 0.8% water agar, which was then overlaid onto solidified nutrient agar plates to form a bacterial mixture layer. The tested agrochemicals were diluted to obtain a range of concentrations. A volume of 0.12 mL from each concentration was applied onto sterile filter paper discs (13 mm diameter; Whatman International Ltd., Leeds, UK). The impregnated discs were then placed on the agar plates previously overlaid with the bacterial mixture layer. Discs treated with sterile distilled water served as negative controls. Each treatment was performed in triplicate. The plates were incubated at 28℃ for 48 h. Following incubation, the diameters of the inhibition zones surrounding each disc were measured to compare bacterial growth inhibition at different agrochemical concentrations.

RESULTS AND DISCUSSION

Bacterial isolation

To investigate the causal agents of tomato wilt, 4 symptomatic tomato plants exhibiting wilting symptoms were randomly collected from a field. Bacterial isolation was performed from discolored vascular tissues of the stems. From one of the tomato samples, yellowish colonies were obtained on nutrient agar. Three representative yellowish isolates were selected and designated as TW01, TW02, and TW03. In contrast, white bacterial colonies were isolated

from the remaining 3 tomato samples. One white colony was selected from each sample, purified, and subsequently designated as TW04, TW05, and TW06 for further identification analyses.

Biolog identification system

The 6 tested bacterial isolates were analyzed using the Biolog identification system. The TW01, TW02, and TW03 isolates were cultured on Biolog GENIII Microplates to assess their physiological and biochemical characteristics. The experimental data were analyzed and compared with the Biolog MicroLog^{TM} 3 ver. 5.2.2 Database. The results indicated that TW01, TW02, and TW03 were identified as *Ps. corrugata* with similarity values of 0.726, 0.682, and 0.667, respectively. TW04, TW05, and TW06 isolates were identified as *Pe. carotovorum* subsp. *carotovorum* with similarity values of 0.661, 0.626, and 0.625, respectively, all greater than the system's predefined threshold value of 0.5.

Pe. carotovorum subsp. *carotovorum* (formerly *Erwinia carotovorum* subsp. *carotovorum*) is an important phytopathogenic bacterium in Taiwan. It has a broad host range and infects various vegetable crops. In tomatoes, it causes bacterial stem rot, which leads to plant wilting (Hseu *et al*. 2003). This pathogen is already established and prevalent in Taiwan. However, Biolog analysis identified strains TW01, TW02, and TW03 as *Ps. corrugata*. According to international literature, *Ps. corrugata* was first reported in 1978 as the causal agent of tomato pith necrosis (Scarlett *et al*. 1978) and has since been reported worldwide, including in African countries such as Tanzania (Black *et al*. 1999); Asian countries such as India (Parvateesam & Shuchita Verma 1992; Trivedi *et al*. 2008) and Japan (Kaji *et al*. 1989; Morohoshi *et al*. 2025); North American countries such as the United States (Powell *et al*. 2013) and Mexico (Rodríguez-Alvarado *et al*. 2002); European countries such as Greece (Buonaurio *et al*. 1993) and Italy (Catara *et al*. 1997); Oceania, including New Zealand (Clark & Watson 1986); and South American countries such as Argentina (Alippi *et al*. 1993) and Brazil (Clark & Watson 1986). Nevertheless, there have been no reports of *Ps. corrugata* infecting crops in Taiwan. Therefore, this study aims to perform further nucleic acid analysis and physiological and biochemical tests on bacterial isolates TW01, TW02, and TW03 to clarify their taxonomic status.

Physiological and biochemical analysis

Physiological and biochemical characterization of the tested bacterial isolates, TW01, TW02, and TW03, was conducted in duplicate, and identical results were obtained. All 3 isolates were Gram-negative, oxidized glucose, and produced yellowish colonies with diffusible pale-yellow pigment on nutrient agar. No fluorescent pigment production was observed on King's B medium. When grown on crystal violet pectate (CVP) agar, the isolates did not form cavities. The isolates were capable of producing levan and were positive for oxidase and arginine dihydrolase activities. They did not cause soft rot on potato slices but elicited a hypersensitive reaction on tobacco leaves. Additionally, the isolates liquefied gelatin and grow at 37℃. Carbon utilization tests indicated that the isolates could metabolize mannitol, trehalose, sucrose, and D-arabinose, but not 2-ketogluconate, meso-tartrate, histamine, benzoate, sorbitol, and L-rhamnose, as summarized in Table 1.

The physiological and biochemical characteristics of the 3 tested bacterial isolates were largely consistent with those reported for *Ps. corrugata* by Schaad *et al*. (2001) and Catara *et al*. (2002), except for their ability to synthesize levan, indicating minor physiological and biochemical differences between the domestic and foreign isolates. Such variation in levan production has also been observed in other phytopathogenic bacteria belonging to the genus *Pseudomonas*, such as *Ps. viridiflava* and *Ps. savastanoi* (González *et al*. 2003; Marchi *et al*. 2005). Furthermore, *Ps. corrugata* was previously divided into 2 groups (Catara *et al*. 2000), one of which was later recognized as a distinct

Table 1. Physiological and biochemical analysis on tomato pith necrosis pathogen.

Characters	Strains from tomato	*Pseudomonas corrugata*[z]
Gram reaction	G (−)	G (−)[y]
O/F[y] test	O	O
Fluorescent pigment on KB[x]	−	−
Levan	+	−
Oxidase	+	+
Pectolytic activity	−	−
Arginine dihydrolase	+	+
Tobacco hypersensitivity reaction	+	V
Gelatin hydrolysis	+	+
Growth at 37℃	+	+
Utilization of:		
2-ketogluconate	−	−
Mannitol	+	+
Benzoate	−	−
Sorbitol	−	−
Trehalose	+	+
Sucrose	+	+
Meso-tartrate	−	−
D-arabinose	+	+
L-rhamnose	−	−
Histamine	−	−

[z] Data are from (Schaad *et al*. 2001; Catara *et al*. 2002).
[y] G (−): Gram negative; O: oxidative; F: fermentation; +: positive; −: negative; V: between 21–79% of strains positive.
[x] KB: King's B medium.

species, *Ps. mediterranea* (Catara *et al.* 2000, 2002). *Ps. mediterranea* has been reported to cause bacterial pith necrosis of tomato in Taiwan (Tsai *et al*. 2018), whereas no reports exist of *Ps. corrugata* infecting tomato in Taiwan. Although the 2 species share highly similar physiological and biochemical profiles, a major distinction lies in their utilization of 3 substrates: *Ps. mediterranea* can utilize meso-tartrate, histamine, and 2-ketogluconate, whereas *Ps. corrugata* cannot. In the present study, the tested bacterial isolates failed to utilize meso-tartrate, histamine, and 2-ketogluconate, indicating that their physiological and biochemical characteristics are more consistent with *Ps. corrugata* rather than *Ps. mediterranea*, which is already present in Taiwan.

16S rRNA and *gyrB* gene sequence analysis

To further ascertain the taxonomic position of the isolated bacteria, genomic DNA from the test bacterial isolates TW01, TW02, and TW03 was subjected to PCR amplification using the universal bacterial primer pair f8/r1510 for the 16S rRNA gene and UP1-1/UP-2r primer pair for the *gyrB* gene. All tested isolates yielded approximately 1500-bp DNA fragment of the 16S rRNA gene and 1200-bp DNA fragment of the *gyrB* gene. The fragments were cloned and sequenced, revealing that the 16S rRNA and *gyrB* gene sequences of TW01, TW02, and TW03 were identical, with 100% sequence similarity among the 3 bacterial isolates. Using TW01 as the representative bacterial isolate, the 16S rRNA and *gyrB* gene sequences were deposited in the GenBank database under accession numbers PX475021 and PX555835, respectively. Basic Local Alignment Search Tool (BLAST) analysis against the NCBI nucleotide database showed that the 16S rRNA gene sequence of TW01 shared 100% identity with *Ps. corrugata* strains (GenBank accession numbers LT629798.1, CP014262.1, CP102175.1), and *gyrB* gene sequences of TW01 also shared 99–100% identity with *Ps. corrugata* strains (GenBank accession numbers CP102177.1, OZ350621.1, LT629798.1) indicating a high degree of similarity between the tested bacterial isolates and *Ps. corrugata*.

Specific primer pair set analysis

To rapidly distinguish whether the bacterial isolates belonged to *Ps. corrugata* or to closely related species such as *Ps. mediterranea*, which was previously classified within the *Ps. corrugata* group, species-specific primer pairs PC5/1 and PC5/2 for *Ps. corrugata* and PC1/1 and PC1/2 for *Ps. mediterranea* were used for PCR amplification. Electrophoretic analysis of the PCR products revealed that the *Ps. corrugata*-specific primers PC5/1 and PC5/2 successfully amplified the expected ~1,100 bp DNA fragment from the 3 tested bacterial isolates

TW01, TW02, and TW03, as well as from the reference strain *Ps. corrugata* DSM 7228, but not from the reference strain *Ps. mediterranea* DSM 16733 (Fig. 2A). Conversely, PCR with the *Ps. mediterranea*-specific primers PC1/1 and PC1/2 yielded the expected ~600 bp DNA fragment from the reference strain *Ps. mediterranea* DSM 16733, but not from the 3 tested bacterial isolates and the *Ps. corrugata* reference strain DSM 7228 (Fig. 2B). These results indicate that the bacterial isolates obtained in this study are *Ps. corrugata*, rather than *Ps. mediterranea*, which has been previously reported in Taiwan.

Whole-genome DNA sequence analysis of the bacterial isolates

To confirm the taxonomic affiliation of bacterial isolate TW01, next-generation sequencing was performed, and the genome was assembled with Velvet assembly software. The resulting draft genome sequence of TW01 (GenBank accession number SUB15775757) was 6,358,395 bp in length, comprising 219 contigs, with an N50 of 68,745 bp and an average sequencing coverage of 334×. Comparative genomic analyses showed that TW01 shared dDDH values of 96.2% and 96.1% with the *Ps. corrugata* type strains NCPPB 2445 and DSM 7228, respectively. Similarly, the ANI values between TW01 and the type strains NCPPB 2445 and DSM 7228 were 99.46% and 99.45%, respectively. Because the dDDH and ANI values between TW01 and the *Ps. corrugata* type strains NCPPB 2445 and DSM7228 were higher than the accepted cut-off thresholds (70% for dDDH and 95–96% for ANI) for prokaryotic species delineation (Goris *et al*. 2007; Richter & Rosselló-Móra 2009; Meier-Kolthoff *et al*. 2013), TW01 was accordingly identified as *Ps. corrugata*.

Koch's postulates test

Tomato plants cv. 'Hong Fan' inoculated with bacterial isolates TW01, TW02, and TW03 by stem puncture developed wilting on lateral branches 14 d post-inoculation (Fig. 3B). Longitudinal stem sections revealed bacterial spread along the vascular bundles, resulting in vascular browning (Fig. 3C) and, in more severe cases, necrosis of the pith tissues (Fig. 3D). As the disease progressed, inoculated plants exhibited stunted growth, followed by chlorosis and decline within 2–3 mo. In contrast, sterile water-inoculated control plants remained symptomless (Fig. 3A). The inoculated bacteria were consistently re-isolated from symptomatic tissues, and molecular analysis confirmed they were identical to the original isolates, thereby fulfilling Koch's postulates.

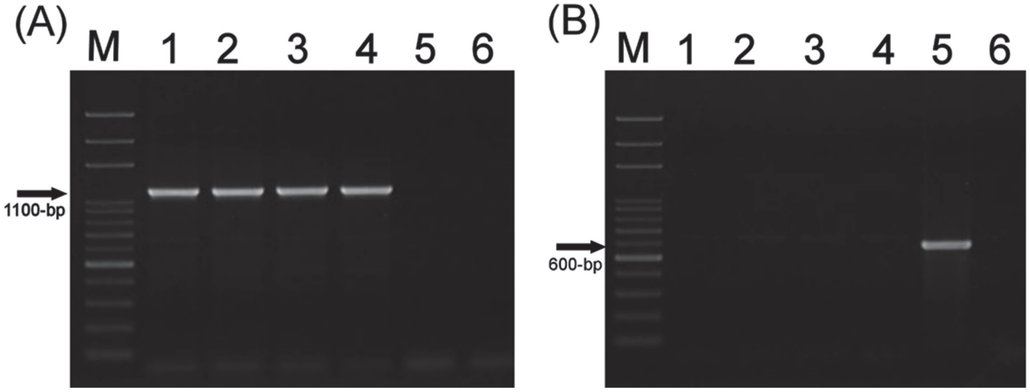

Fig. 2. Electrophoresis analysis of polymerase chain reaction (PCR) products for the identification of tomato pith necrosis pathogens. (A) PCR amplification with *Pseudomonas corrugata* specific primer PC5/1 and PC5/2; (B) PCR amplification with *Ps. mediterranea* PC1/1 and PC1/2. Lane M: 100 bp DNA marker; Lane 1–3: DNA from bacterial isolates TW01, TW02, and TW03; Lane 4: DNA from *Ps. corrugata* (DSM7228); Lane 5: DNA from *Ps. mediterranea* (DSM16733); Lane 6: no template control.

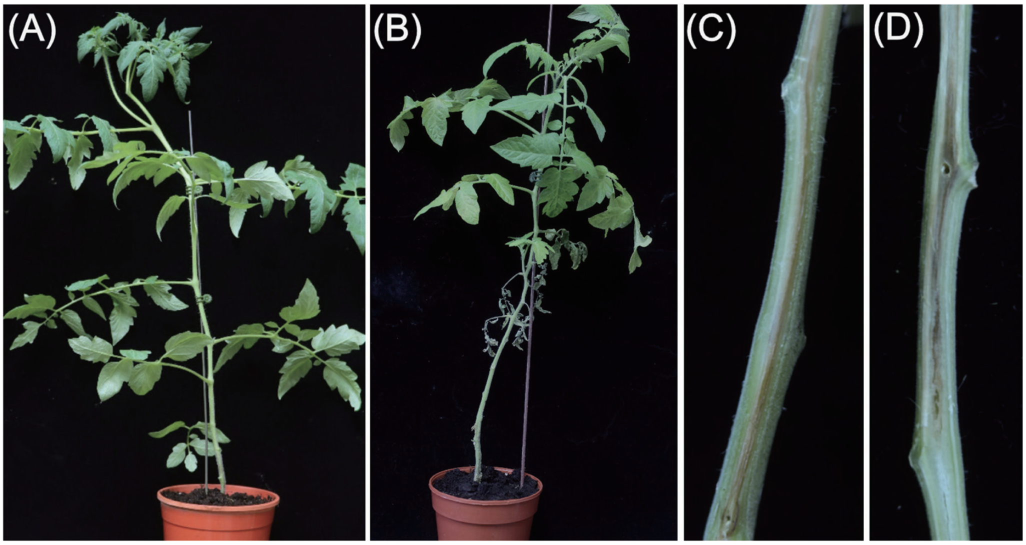

Fig. 3. Symptoms of the tomato plants 'Hong Fan' after inoculation with the isolated bacteria. (A) Tomato plants inoculated with sterile distilled water, serving as the control, showed no symptoms; (B) The lateral branches of tomato plants exhibited wilting near the inoculation sites; (C) Browning of stem vascular bundles in inoculated plants; (D) Necrosis of pith tissues in severely infected stems.

In vitro agrochemicals susceptibility test

The inhibitory effects of 11 commercial agrochemicals at 3 different concentrations against bacterial isolates TW01 and TW03 were evaluated on agar medium using the paper disc diffusion method. After 2 d of incubation, the diameters of inhibition zones (excluding the 13 mm diameter of the paper discs) were measured. Each was performed in duplicate, and the results were consistent; therefore, the data from one representative experiment are presented in Table 2. Among the tested agrochemicals, 4 produced inhibition zones against both bacterial isolates: streptomycin, streptomycin + tetracycline, thiophanate methyl + streptomycin, and kasugamycin. In contrast, the other 7 agrochemicals- validamycin, fosetyl-aluminium + isotianil, kasugamycin + copper oxychloride, copper hydroxide, tribasic copper sulfate, oxolinic acid, and mancozeb- showed no inhibitory zone at the tested concentrations. For bacterial isolate TW01, the largest inhibition zones were observed with streptomycin (500× dilution) and streptomycin + tetracycline (500× dilution), with diameters of 3.00–3.27 mm, followed by streptomycin (1,000× dilution) and thiophanate methyl + streptomycin (500× dilution), with diameters of 2.20–2.30 mm. For bacterial isolate TW03, the largest inhibition zones were observed with streptomycin (500× dilution), streptomycin + tetracycline (500× dilution), and thiophanate methyl + streptomycin (500× dilution) with diameters of 3.77–4.30 mm, followed by streptomycin (1,000× dilution), streptomycin + tetracycline (1,000× dilution), thiophanate methyl + streptomycin (1,000× dilution), and kasugamycin (125× dilution), with diameters of 2.50–3.03 mm.

Among the 4 agrochemicals that inhibited bacterial growth, 3 also produced inhibition zones against both bacterial isolates at the recommended product concentration, namely streptomycin (1,000× dilution), thiophanate methyl + streptomycin (1,000× dilution), and streptomycin + tetracycline (1,000× dilution). In contrast, copper-based agrochemicals failed to generate inhibition zones at any of the 3 tested concentrations, indicating that their antibacterial activities were ineffective in the range above and below 1/2 of the recommended concentration.

Table 2. Growth inhibition of *Pseudomonas courugata* TW01 and TW03 by various agrochemicals in different concentrations.

Chemical[z]	Dilution fold	Inhibition zone (mm in diameter)	
		TW01	TW03
Streptomycin (12.5% SL)	500	3.27 ± 0.10 a[y]	4.30 ± 0.38 a
	1,000	2.30 ± 0.15 b	2.90 ± 0.26 b
	1,500	0 e	1.67 ± 0.34 d
Streptomycin + Tetracycline (10% SP)	500	3.00 ± 0.10 a	4.07 ± 0.17 ab
	1,000	1.80 ± 0.06 c	2.80 ± 0.06 bc
	1,500	0.63 ± 0.13 e	1.57 ± 0.20 d
Thiophanate methyl + Streptomycin (68.8% WP)	500	2.20 ± 0.35 b	3.77 ± 0.18 ab
	1,000	1.33 ± 0.09 d	3.03 ± 0.03 b
	1,500	0.53 ± 0.27 e	2.13 ± 0.15 c
Kasugamycin (2.0%WP)	125	0.47 ± 0.47 e	2.50 ± 0.20 bc
	250	0 f	0 e
	375	0 f	0 e
Kasugamycin + Copper oxychloride (81.3% WP)	500	0 f	0 e
	1,000	0 f	0 e
	1,500	0 f	0 e
Validamycin (20%SL)	600	0 f	0 e
	1,200	0 f	0 e
	1,800	0 f	0 e
Fosetyl-aluminium + Isotianil (77%WG)	350	0 f	0 e
	700	0 f	0 e
	1,050	0 f	0 e
Oxolinic acid (20% WP)	500	0 f	0 e
	1,000	0 f	0 e
	1,500	0 f	0 e
Copper hydroxide (53.8% WG)	1,000	0 f	0 e
	2,000	0 f	0 e
	3,000	0 f	0 e
Tribasic copper sulfate (27.12% SC)	250	0 f	0 e
	500	0 f	0 e
	750	0 f	0 e
Mancozeb (80.0% WP)	250	0 f	0 e
	500	0 f	0 e
	750	0 f	0 e
LSD		0.34	0.35

[z] SL: soluble concentrate; SP: water soluble powder; WP: wettable powder; WG: water dispersible granules; SC: suspension concentrate; LSD: Fisher's least significant difference.

[y] Mean ± standard error ($n = 3$). Means within each column followed by the same letter(s) are not significantly different at 5% level by LSD test.

According to international literature on agrochemical assays of *Ps. corrugata*, this pathogen exhibits a relatively high tolerance to copper-based compounds (Alippi *et al*. 2003).

Consistently, in the present study, copper-based agrochemicals demonstrated limited inhibitory activity against the tested bacterial isolates at the applied concentrations, which is in agreement with the findings reported by Alippi *et al.* (2003). Since *Ps. corrugata* is newly reported in Taiwan and no local information on agrochemical control is available, this study provides an initial evaluation of agrochemicals against the pathogen. Four agrochemicals were identified as capable of producing inhibition zones and suppressing bacterial growth, suggesting their potential for disease management. These agrochemicals and the concentrations that produced inhibition zones *in vitro* may serve as preliminary references for future field trials or seed disinfection assays.

CONCLUSIONS

In 2014, tomato plants exhibiting wilting symptoms were observed in cultivation fields located in the Waipu District, Taichung City, Taiwan. From symptomatic plants, a yellowish bacterial isolate was obtained from one diseased sample. This bacterial isolate was identified as *Ps. corrugata* and subsequently confirmed as the causal agent by fulfilling Koch's postulates. Inoculated tomato plants developed vascular browning and pith necrosis, thereby verifying that the bacterium is the causal pathogen of tomato pith necrosis, as previously described by Scarlett *et al.* (1978).

Tomato pith necrosis is caused by several pathogenic species of the genus *Pseudomonas*. In Taiwan, *Ps. viridiflava* and *Ps. mediterranea* have previously been reported to infect tomato and induce pith necrosis (Tsai *et al.* 2016, 2018). In contrast, the pathogen isolated from symptomatic tomato plants in the Waipu District, Taichung City, was identified as *Ps. corrugata*, a species distinct from those previously reported in Taiwan. To date, *Ps. corrugata* has not been reported as a causal agent of tomato infection in Taiwan; this is the first report of tomato infection caused by *Ps. corrugata* in Taiwan. After the disease occurred in that field, tomatoes were no longer cultivated, and the disease has not reappeared since.

Regarding transmission, results from the inoculation trials indicate that *Ps. corrugata* can infect tomato plants through minor stem wounds. Thus, in the field, the pathogen may enter and colonize stems via pruning or other mechanical injuries. Previous reports have also shown that *Ps. corrugata* can survive in soil (Moura *et al.* 2012), infected fruits, and seeds (Cirvilleri *et al.* 2008). Therefore, effective management of this disease should include disinfecting pruning tools, protection of pruning wounds, selection of disease-free fields for planting, avoidance of irrigation using water from infected fields, timely removal of diseased plants, and use of pathogen-free seeds.

For agrochemical control, 4 commercially available agrochemicals were preliminarily screened *in vitro* in this study. Within the tested concentration ranges, these agrochemicals inhibited the growth of the pathogen on culture media. The identified agrochemicals and their effective concentrations may serve as references for future field trials or seed treatment experiments.

ACKNOWLEDGMENTS

We thank the Animal and Plant Health Inspection Agency, Ministry of Agriculture, for assisting in the introduction of the reference strains *Ps. corrugata* DSM 7228 (Import Permit No.103-B-004) and *Ps. mediterranea* DSM 16733 (Import Permit No. 104-B-005E) used in this study.

REFERENCES

Adaskaveg, J. E. and R. B. Hine. 1985. Copper tolerance and zinc sensitivity of Mexican strains of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of pepper. Plant Dis. 69:993–996. doi:10.1094/PD-69-993

Alippi, A. M., E. Dal Bo, L. B. Ronco, M. V. López, A. C. López, and O. M. Aguilar. 2003. *Pseudomonas* populations causing pith necrosis of tomato and pepper in Argentina are highly diverse. Plant Pathol.

52:287–302. doi:10.1046/j.1365-3059.2003.00850.x

Alippi, A. M. and A. C. López. 2010. First report of *Pseudomonas mediterranea* causing tomato pith necrosis in Argentina. Plant Pathol. 59:1163. doi:10.1111/j.1365-3059.2010.02291.x

Alippi, A. M., L. Ronco, and H. E. Alippi. 1993. Tomato pith necrosis caused by *Pseudomonas corrugata* in Argentina. Plant Dis. 77:428. doi:10.1094/PD-77-0428C

Aysan, Y., N. Yildiz, and F. Yucel. 2004. Identification of *Pseudomonas viridiflava* on tomato by traditional methods and enzyme-linked immunosorbent assay. Phytoparasitica 32:146–153. doi:10.1007/BF02979780

Bella, P. and V. Catara. 2010. Occurrence of tomato pith necrosis caused by *Pseudomonas marginalis* in Italy. Plant Pathol. 59:402. doi:10.1111/j.1365-3059.2009.02185.x

Black, R., S. Seal, Z. Abubakar, R. Nono-Womdim, and I. Swai. 1999. Wilt pathogens of Solanaceae in Tanzania: *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas corrugata*, and *Ralstonia solanacearum*. Plant Dis. 83:1070. doi:10.1094/PDIS.1999.83.11.1070A

Buonaurio, R., A. Karofilakis, and M. Scortichini. 1993. Occurrence of *Pseudomonas corrugata* roberts et scarlett on tomato plants in Crete. Phytopathol. Mediterr. 32:245–246.

Caruso, A., G. Licciardello, R. La Rosa, V. Catara, and P. Bella. 2016. Mixed infection of *Pectobacterium carotovorum* subsp. *carotovorum* and *P. carotovorum* subsp. *brasiliensis* in tomato stem rot in Italy. J. Plant Pathol. 98:661–665. doi:10.4454/JPP.V98I3.062

Catara, V., D. Arnold, G. Cirvilleri, and A. Vivian. 2000. Specific oligonucleotide primers for the rapid identification and detection of the agent of tomato pith necrosis, *Pseudomonas corrugata*, by PCR amplification: Evidence for two distinct genomic groups. Eur. J. Plant Pathol. 106:753–762. doi:10.1023/A:1026507423848

Catara, V., L. Gardan, and M. M. Lopez. 1997. Phenotypic heterogeneity of *Pseudomonas corrugata* strains from southern Italy. J. Appl. Microbiol. 83:576–586. doi:10.1046/j.1365-2672.1997.00268.x

Catara, V., L. Sutra, A. Morineau, W. Achouak, R. Christen, and L. Gardan. 2002. Phenotypic and genomic evidence for the revision of *Pseudomonas corrugata* and proposal of *Pseudomonas mediterranea* sp. nov. Intl. J. Syst. Evol. Microbiol. 52:1749–1758. doi:10.1099/00207713-52-5-1749

Cirvilleri, G., P. Bella, R. La Rosa, and V. Catara. 2008. Internalization and survival of *Pseudomonas corrugata* from flowers to fruits and seeds of tomato plants. p.73–79. *in*: *Pseudomonas syringae* Pathovars and Related Pathogens- Identification, Epidemiology and Genomics. (Fatmi, M., A. Collmer, N. Sante Iacobellis, J. W. Mansfield, J. Murillo, N. W. Schaad, and M. Ullrich, eds.) Springer. Dordrecht, The Netherlands. 433 pp. doi:10.1007/978-1-4020-6901-7_8

Clark, R. G. and D. R. W. Watson. 1986. New plant disease record in New Zealand: Tomato pith necrosis caused by *Pseudomonas corrugata*. N. Z. J. Agric. Res. 29:105–109. doi:10.1080/00288233.1986.10417981

González, A. J., M. R. Rodicio, and M. C. Mendoza. 2003. Identification of an emergent and atypical *Pseudomonas viridiflava* lineage causing bacteriosis in plants of agronomic importance in a Spanish region. Appl. Environ. Microbiol. 69:2936–2941. doi:10.1128/AEM.69.5.2936-2941.2003

Goris, J., K. T. Konstantinidis, J. A. Klappenbach, T. Coenye, P. Vandamme, and J. M. Tiedje. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Intl. J. Syst. Evol. Microbiol. 57:81–91. doi:10.1099/ijs.0.64483-0

Hseu, S. H., C. Y. Lin, and T. C. Sung. 2003. Bacterial stem rot of tomato caused by *Erwinia carotovora* subsp. *carotovora*. Plant Prot. Bull. 45:257–262. (in Chinese with English abstract) doi:10.6715/PPB.200309_45(3).0008

Kaji, K., K. Tairako, S. Takechi, and Y. Takikawa. 1989. Occurrence of pith necrosis of tomato caused by *Pseudomonas corrugata* Roberts and Scarlett 1981 in Fukushima Prefecture. Ann. Rept. Plant Prot. North Japan 40:52–55. (in Japanese) doi:10.11455/kitanihon1966.1989.52

Lee, I., Y. O. Kim, S. C. Park, and J. Chun. 2016. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. Intl. J. Syst. Evol. Microbiol. 66:1100–1103. doi:10.1099/ijsem.0.000760

Lipson, D. A. and S. K. Schmidt. 2004. Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. Appl. Environ. Microbiol. 70:2867–2879. doi:10.1128/AEM.70.5.2867-2879.2004

Marchi, G., C. Viti, L. Giovannetti, and G. Surico. 2005. Spread of levan-positive populations of *Pseudomonas savastanoi* pv. *savastanoi*, the causal agent of olive knot, in central Italy. Eur. J. Plant Pathol. 112:101–112. doi:10.1007/s10658-005-0804-0

Meier-Kolthoff, J. P., A. F. Auch, H. P. Klenk, and M. Göker. 2013. Genome sequence-based species

delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. doi:10.1186/1471-2105-14-60

Molan, Y. and Y. Ibrahim. 2007. First report of tomato (*Lycopersicon esculentum*) pith necrosis caused by *Pseudomonas fluorescens* and *P. corrugata* in the Kingdom of Saudi Arabia. Plant Dis. 91:110. doi:10.1094/PD-91-0110B

Morohoshi, T., X. Xie, T. Suzuki, and N. Someya. 2025. Comparative analysis of quorum-sensing systems that control pathogenicity in three phylogenetic groups of *Pseudomonas* isolated from tomato pith necrosis in Japan. Biosci. Biotechnol. Biochem. 89:1509–1516. doi:10.1093/bbb/zbaf100

Moura, L., I. Queiroz, I. Mourão, and L. M. Brito. 2012. Survival and genotypic variation of *Pseudomonas corrugata* and *P. mediterranea* in response to soil solarization and biofumigation. p.553–558. *in*: XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on Organic Horticulture: Productivity and Sustainability. August 22–27, 2010. Lisbon, Portugal. International Society for Horticultural Science. Leuven, Belgium. doi:10.17660/ActaHortic.2012.933.72

Moura, M. L., M. A. Jacques, L. M. Brito, I. M. Mourão, and J. Duclos. 2005. Tomato pith necrosis (TPN) caused by *P. corrugata* and *P. mediterranea*: Severity of damages and crop loss assessment. Acta Hortic. 695:365–372. doi:10.17660/ActaHortic.2005.695.45

Parvateesam, M. and S. V. Shuchita Verma. 1992. Enzyme production in *Pseudomonas* isolated from rotten vegetables. Indian J. Mycol. Plant Pathol. 22:197–198.

Phiri, T. M., G. Bhattarai, K. E. Chiwina, Q. Fan, H. Xiong, I. Alatawi, ... A. Shi. 2024. An evaluation of bacterial wilt (*Ralstonia solanacearum*) resistance in a set of tomato germplasm from the United States Department of Agriculture. Agronomy 14:350. doi:10.3390/agronomy14020350

Powell, M., B. Gundersen, C. A. Miles, J. L. Humann, B. K. Schroeder, and D. A. Inglis. 2013. First report of tomato pith necrosis (*Pseudomonas corrugata*) on tomato (*Solanum lycopersicum*) in Washington. Plant Dis. 97:1381. doi:10.1094/PDIS-03-13-0265-PDN

Richter, M. and R. Rosselló-Móra. 2009. Shifting the genomic gold standard for the prokaryotic species definition. Proc. Natl. Acad. Sci. U.S.A. 106:19126–19131. doi:10.1073/pnas.0906412106

Rodríguez-Alvarado, G., J. Holguín-Peña, N. Ochoa-Álvarez, S. P. Fernández-Pavía, and J. A. Geraldo-Verdugo. 2002. *Pseudomonas corrugata* causing pith necrosis on tomato plants in Baja California Sur, México. Plant Dis. 86:563. doi:10.1094/PDIS.2002.86.5.563B

Ruan, H., N. Shi, Y. Du, F. Chen, X. Yang, L. Gan, and Y. Dai. 2019. First report of *Pseudomonas cichorii* causing tomato pith necrosis in Fujian Province, China. Plant Dis. 103:145. doi:10.1094/PDIS-06-18-0959-PDN

Saygili, H., Y. Aysan, F. Sahin, N. Ustun, and M. Mirik. 2004. Occurrence of pith necrosis caused by *Pseudomonas fluorescens* on tomato plants in Turkey. Plant Pathol. 53:803. doi:10.1111/j.1365-3059.2004.01092.x

Scarlett, C. M., J. T. Fletcher, P. Roberts, and R. A. Lelliott. 1978. Tomato pith necrosis caused by *Pseudomonas corrugata* n. sp. Ann. Appl. Biol. 88:105–114. doi:10.1111/j.1744-7348.1978.tb00684.x

Schaad, N. W., J. B. Jones, and W. Chun. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3rd ed. American Phytopathological Society. St. Paul, MN. 373 pp.

Sen, Y., J. van der Wolf, R. G. F. Visser, and S. van Heusden. 2015. Bacterial canker of tomato: Current knowledge of detection, management, resistance, and interactions. Plant Dis. 99:4–13. doi:10.1094/PDIS-05-14-0499-FE

Trivedi, P., A. Pandey, and L. M. S. Palni. 2008. *In vitro* evaluation of antagonistic properties of *Pseudomonas corrugata*. Microbiol. Res. 163:329–336. doi:10.1016/j.micres.2006.06.007

Tsai, C. H., P. J. Ann, Y. S. Lu, M. D. Chen, S. L. Hwang, and T. H. Hung. 2016. Occurrence of pith necrosis of tomato caused by *Pseudomonas viridiflava* in Taiwan. J. Taiwan Agric. Res. 65:269–277. (in Chinese with English abstract) doi:10.6156/JTAR/2016.06503.04

Tsai, C. H., P. J. Ann, Y. S. Lu, S. L. Hwang, and T. H. Hung. 2018. Occurrence of pith necrosis of tomato caused by *Pseudomonas mediterranea* in Taiwan. J. Taiwan Agric. Res. 67:16–27. (in Chinese with English abstract) doi:10.6156/JTAR.201803_67(1).0003

Wang, H., M. Qi, and A. J. Cutler. 1993. A simple method of preparing plants samples for PCR. Nucleic Acids Res. 21:4153–4154. doi:10.1093/nar/21.17.4153

Yamamoto, S. and S. Harayama. 1995. PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. Appl. Environ. Microbiol. 61:1104–1109. doi:10.1128/aem.61.3.1104-1109.1995

番茄髓壞疽病之病原菌 *Pseudomonas corrugata* 之鑑定與藥劑篩選

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摘要

蔡佳欣、黃淑苓、林玟珠、古喬羽、洪挺軒。2026。番茄髓壞疽病之病原菌 *Pseudomonas corrugata* 之鑑定與藥劑篩選。台灣農業研究 75(2):293–306。

在 2014 年，臺中市外埔區 1 處番茄田植株部分植株出現生長緩慢，並且果實易有落果的情形，罹病植株莖部表皮黑褐化，縱切莖部發現維管束褐化且蔓延，將受害莖部組織在光學顯微鏡下鏡檢具有大量細菌湧出，疑似細菌性病害。從罹病株分離病原菌，在營養培養基分離出番茄細菌性莖腐細菌 *Pectobacterium carotovorum* subsp. *carotovorum* 之外，另外分離出 1 種未知的淡黃色細菌，該未知細菌，經 Biolog 鑑定系統分析、細菌生理生化特性分析、專一性引子對鑑定以及細菌基因序列分析，將該菌鑑定為 *Pseudomonas corrugata*，將其接種至番茄植株，在莖部出現維管束褐化蔓延病徵，與田間所見相同，嚴重感染之植株，髓部可觀察到壞疽病徵產生，並可再分離出該細菌，完成科霍氏法則。此為 *P. corrugata* 感染番茄在臺灣之首次報導。為防治此細菌，在培養基進行市售農藥篩選試驗，以鏈黴素、鏈四環黴素及多保鏈黴素具有明顯的抑制細菌生長效果，並形成明顯的抑制圈。該田區之後未再繼續種植番茄，未再發生該病害。

關鍵詞：番茄、髓壞疽病、*Pseudomonas corrugata*、藥劑篩選。

投稿日期：2025 年 9 月 11 日；接受日期：2026 年 1 月 28 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):307–321 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0010 研究報告

分離自杏鮑菇之青黴菌 (*Penicillium sumatraense*) 鑑定與藥劑防治評估

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摘要

蔡承佑、余祥萱、呂昀陞。2026。分離自杏鮑菇之青黴菌 (*Penicillium sumatraense*) 鑑定與藥劑防治評估。台灣農業研究 75(2):307–321。

杏鮑菇 (*Pleurotus eryngii* (DC.) Quél.) 為臺灣重要商業栽培菇種，年平均產量近 2.6 萬公噸，產值約 20 億元，主要產區包含臺中市與彰化縣等地。2024 年於臺中新社區發現，杏鮑菇於出菇階段，太空包中菌絲出現褐化並產生大量褐色菇水，嚴重影響其子實體品質，且在太空包表面菌絲處可見青黴菌之徵狀。本研究對該菌進行分離，並將純化之菌株與杏鮑菇進行培養基對峙培養，結果顯示靠近分離菌株之杏鮑菇菌絲長度顯著受到影響，顯見此菌株可影響杏鮑菇菌絲之生長。進一步鏡檢分離菌株之特徵，確認該菌具有長度 155–203 µm、雙輪生 (biverticillate) 之帚狀分生孢子梗 (conidiophore)，其上著生 3–5 個梗基 (metulae)，每個梗基上接有 3–5 個壺狀 (ampulliform) 之產孢瓶梗 (phialide)；末端產孢，分生孢子為透明、橢圓形至圓形。根據形態特徵與內轉錄間隔區 (internal transcribed spacer; ITS) 序列親緣演化樹分析，將分離菌株鑑定為 *Penicillium sumatraense* Svilv. (菌株代號：TARI-PeP01)。菌絲生長溫度試驗結果則顯示，TARI-PeP01 可生長於 16–28℃，並於 28℃時具最大菌落直徑；溫度提升至 32℃時，其菌落長度則顯著降低。為利後續防治策略擬定，本研究亦進行藥劑篩選試驗，結果顯示稀釋 2,000 倍之 50% 撲克拉錳 (可濕性粉劑) 與稀釋 5,000 倍之 35% 撲克拉 (乳劑)，可分別抑制 32.16 與 24.43% TARI-PeP01 之菌落生長，兩藥劑又皆以稀釋 1,000 倍可顯著抑制菌絲生長，顯示該兩種藥劑具防治之潛力。本研究為臺灣首篇針對杏鮑菇之青黴菌進行鑑定與防治之報告，而所篩選出具防治潛力之藥劑，可作為後續防患此真菌之參考依據。未來除深入探討 *P. sumatraense* 對杏鮑菇之生理影響外，也將持續調查其他青黴菌對菇類產業之影響。

關鍵詞：青黴菌、杏鮑菇、撲克拉。

前言

杏鮑菇 (*Pleurotus eryngii* (DC.) Quél.) 為側耳科大型真菌，原產於南歐、地中海區域、中東與北非，其菌傘與菌柄質地肉質，富含高蛋白質、多醣及礦物質，且脂肪含量低 (Yan *et al*. 2002)，更具有多種健康功效，如免疫調節、降血壓、血脂與血糖、抗腫瘤及延緩骨質疏鬆等功能 (Lindequist *et al*. 2005)，因此被視為天然且具營養保健特性的食用菇。現今市售之杏鮑菇係由農業部農業試驗所 (簡稱農試所) 選育後，形成之商業品種，因此臺灣為最早種植與生產杏鮑菇的國家 (Peng *et al*. 2001)。目前杏鮑菇每年平均產量可達 2.6 萬公噸，產值約為 20 億元，係屬臺灣產量第 3 大之菇種，產地主要分布於臺中市霧峰區、新社區；彰化縣和美鎮、埔心鄉；以及南投縣魚池鄉等中部地區 (Chen *et al*. 2016)。

投稿日期：2025 年 8 月 14 日；接受日期：2025 年 12 月 15 日。
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杏鮑菇培育過程如受環境中病原菌感染或雜菌污染，會導致生長週期延長或產量下降等問題，故此兩者皆為杏鮑菇生產的重要限制因子 (Ruan *et al*. 2008)。杏鮑菇栽培期間已知可造成危害之病原菌包含病毒、細菌及真菌等，其中病毒 *P. eryngii* Spherical Virus (PeSV) 之寄生可造成杏鮑菇菌絲生長緩慢與子實體畸形 (Ro *et al*. 2007)；細菌病害則包含由 *Pseudomonas tolaasii* 造成之細菌性褐斑病 (González *et al*. 2009)、*Pantoea* sp. 引起之細菌性軟腐病 (Kim *et al*. 2007) 及 *Burkholderia gladioli* 引起之細菌性腐敗病 (Lin *et al*. 2010) 等；真菌病害則有由 *Cladobotryum varium*、*C. mycophilum* 引起之杏鮑菇白黴病 (Arima & Suyama 2000; Back *et al*. 2012)、*Gliocladium roseum* 引起之杏鮑菇褐斑病 (Chen & Huang 2004)、木黴菌屬 (*Trichoderma*) 等可造成菌絲傷害之病原 (Šašić Zorić *et al*. 2023)。此外競爭菇類菌絲之空間與營養的雜菌，如多數青黴菌屬 (*Penicillium*) 與黃麴黴菌 (*Aspergillus*) (Komon-Zelazowska *et al*. 2007)；以及造成其他污染之毛黴菌屬 (*Mucor*)、紅麵包黴屬 (*Neurospora*)、枝孢菌屬 (*Cladosporium*) 及擬莖點黴屬 (*Phomopsis*) 等 (Kim *et al*. 2013)。

木黴菌屬 (*Trichoderma*)、青黴菌屬 (*Penicillium*) 及黃麴黴菌屬 (*Aspergillus*) 等真菌均可污染菇類太空包，並於太空包上形成黃綠色至深綠色之徵狀，故常被通稱為綠黴病。此類菌主要影響菇類菌絲於太空包內之生長，嚴重時甚至導致無法出菇，為菇類產業重要的危害因子。其中青黴菌屬生存環境廣泛，自空氣、土壤、蔬果及食品，甚至是深海水域皆可分離獲得，部分物種更可造成柑橘與蘋果等食物腐壞 (Tzean *et al*. 1994; Visagie *et al*. 2014)，在栽培菇類時也是常見之雜菌。青黴菌之屬名拉丁文意義為 “brush”，係用來描述其分生孢子梗如掃帚狀的分支，本屬真菌過往因物種形態相近，且有性世代與無性世代學名繁多，導致重名與鑑定錯誤問題嚴重，因此 Houbraken *et al*. (2011) 導入分子證據將此屬分類重新建構，並分類至麴菌科 Aspergillaceae (包含 *Aspergillus*、*Penicilliopsis* 及 *Penicillium* 屬等)。目前為有效鑑定與釐清此些相近物種之親緣關係，需配合測試培養基之菌落特徵、菌體顯微形態特徵及菌絲適溫性等基本特性，並輔以多基因分子親緣分析 (multi-locus sequence analysis; MLSA)，可再將本屬細分為 2 個亞屬 (subgenus) *Aspergilloides* 與 *Penicillium* 以及亞屬之下的 25 個 section (Visagie *et al*. 2014)。

本研究於 2024 年在臺中市新社區杏鮑菇栽培場中，發現徵狀疑似為青黴菌之真菌可於開包出菇時之杏鮑菇菌絲表面纏據生長，並使菇包產生褐色菇水現象，嚴重影響杏鮑菇出菇能力，進而導致產量下降。因此本研究即針對受影響之杏鮑菇菌絲處，分離與純化該真菌，並進一步進行形態觀察、親緣分析鑑定、生長特性與防治藥劑之篩選，以作為未來防治此類真菌的參考依據。

材料與方法

供試菌株之分離與保存

以過火消毒之移植針挑取菌絲表面之青黴病徵兆，並放置於 potato dextrose agar (PDA; BD Difco, Franklin Lakes, NJ, USA) 培養基上培養，待菌絲生長後以移植針切取菌絲尖端純化更新 3 次，並將分離菌株編號為 TARI-PeP01。以 PDA 培養 7 日後以 5 mm 打孔器切取之 TARI-PeP01 菌絲邊緣，放置於無菌水中，保存於 24℃。杏鮑菇菌株為農試所菇類研究室保存之杏鮑菇 (*P. eryngii* strain TARI-PE01)。於試驗前，所有供試菌株先以 PDA 於 24℃下進行活化培養 7 日後備用。

青黴菌與杏鮑菇之對峙培養

試驗前先行製備青黴菌之孢子懸浮液，以 Visagie *et al*. (2014) 發表之方法進行修正。將 TARI-PeP01 更新於 malt extract agar (MEA) 培養基 (50 g malt extract (Oxoid, Basingstoke, UK)，1 mL trace elements stock solution (0.5 g $CuSO_4 \cdot 5H_2O$，0.1 g $ZnSO_4 \cdot 7H_2O$，100 mL distilled water (D.W.))，20 g agar，1 L D.W.)，並以 24℃培養 7 日。再以 1 mL 含有 30% (v/v) glycerol、0.05% (v/v) tween 80 與

0.05% (w/v) agar 之無菌水，滴於培養好之 MEA 培養基，並洗下分生孢子，調整成濃度為 10^6 spore mL^{-1} 之孢子懸浮液。定量滴加 2 μL TARI-PeP01 孢子懸浮液於 PDA、MEA 及木屑培養基 (sawdust medium; SDM；85% (w/w) 雜木屑，15% (w/w) 米糠，含水量 65%) 之一側 (距離培養基中心點 1.5 cm 處)，另一側則接種於 PDA 培養 7 日後之 5 mm TARI-PE01 菌絲塊。接種完畢後，培養皿均置於 24℃ 定溫箱中避光培養，於 7 日後，分別測量 TARI-PeP01 與 TARI-PE01 菌落大小直徑。每處理共 5 皿，本試驗進行 3 次重複。

青黴菌之形態鑑定與生長速度測定

菌落形態鑑定使用之培養基係依前人研究之方法進行 (Odds 1991; Tzean *et al*. 1994; Visagie *et al*. 2014; Andrade *et al*. 2021)。分別滴加 2 μL TARI-PeP01 孢子懸浮液 (10^6 spore mL^{-1}) 於高溫高壓滅菌之 PDA、MEA、Sabouraud's agar (SA) 培養基 (40 g glucose，10 g peptone，20 g agar，1 L D.W.)、Czapek yeast autolysate agar (CYA) 培養基 (10 mL Czapek concentrate：30 g $NaNO_3$、5 g KCl、5 g $MgSO_4 \cdot 7H_2O$、0.1 g $FeSO_4 \cdot 7H_2O$，100 mL D.W.)，30 g sucrose，5 g yeast extract (BD Difco, Franklin Lakes, NJ, USA)，1 g K_2HPO_4，1 mL trace elements stock solution，20 g agar，1 L D.W.)、Czapek yeast autolysate agar with sodium chloride (CYAS；CYA 添加 5% (w/v) NaCl)、dichloran 18% glycerol agar (DG18) 培養基 (31.6 g dichloran-glycerol-chloramphenicol-agar (Merck Millipore, Burlington, MA, USA)，1 mL trace elements stock solution，175 mL glycerol，20 g agar，1 L D.W.)、oatmeal agar (OA) 培養基 (30g oatmeal (flakes)，1 mL trace elements stock solution，20 g agar，1 L D.W.)、yeast extract sucrose agar (YES) 培養基 (150 g sucrose，20 g yeast extract，0.5 g $MgSO_4 \cdot 7H_2O$，1 mL trace elements stock solution，20 g agar，885 mL D.W.)、creatine sucrose agar (CREA) 培養基 (30 g sucrose，3 g creatine · H_2O，1 g $K_3PO_4 \cdot 7H_2O$，0.5 g $MgSO_4 \cdot 7H_2O$，0.5 g KCl，0.01 g $FeSO_4 \cdot 7H_2O$，1 mL trace elements stock solution，0.05 g bromocresol purple，20 g agar，1 L D.W.) 共 9 種培養基，各培養基體積固定為 20 mL。接種完成後，為利保持通氣促進產孢，培養皿不以石臘膜密封，放置於 25℃定溫箱中避光培養，其中 CYA 培養基額外置於 30℃與 37℃中避光培養，於第 7 日記錄各培養基之青黴菌菌落正反面形態、顏色及質地等，並量測菌落直徑；此外為觀察本菌於培養基是否產生菌核或有性世代，將 OA 培養皿密封放置於 25℃定溫箱中培養 3 個月。本試驗每處理共 5 皿，並進行 3 次重複。

顯微形態觀察部分，以移植針刮取培養在 MEA 上生長 7–10 日之 TARI-PeP01 菌絲，並懸浮於 60% (v/v) 乳酸 (lactic acid) 水溶液中進行鏡檢觀察，可視情況使用 70% 酒精洗去多餘分生孢子。以具攝影功能之複式顯微鏡 (Axioskop 2, Carl Zeiss, Oberkochen, Germany) 記錄形態特徵並量測 100 個分生孢子 (conidia)、分生孢子梗 (conidiophore) 及壺狀產孢細胞 (phialides) 大小。

溫度對青黴菌生長之影響測定

為測試溫度對青黴菌菌絲生長之影響，分別滴加 2 μL TARI-PeP01 孢子懸浮液於 PDA、MEA 及 SDM 上，分別放置於 16℃、20℃、24℃、28℃及 32℃定溫箱中黑暗培養，於培養 7 日後測量其菌落直徑。每處理共 5 皿，本試驗進行 3 次重複。

青黴菌之核酸序列分析與親緣演化樹

為進一步進行分子鑑定，本研究刮取 MEA 上 TARI-PeP01 菌絲，以 Maelstrom Switch 8 Automated DNA/RNA Purification Platform (TANBEAD company, Taoyuan, Taiwan) 完成真菌總基因體 DNA (total genomic DNA; gDNA) 抽取，萃取之 gDNA 保存於 -20℃，以供後續實驗使用。將 gDNA 1 μL、dH_2O 22 μL、引子對各 1 μL：ITS4 (5'-TCCTCCGCTTATTGATATGC-3') 與 ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') (White *et al*. 1990)、2× Advanced Taq Master Mix Fast-Run™ (ProTECH, Taipei, Taiwan) 25 μL，總反應體積為 50 μL，混合均勻後，

置入自動溫度循環控制器 (ABI GeneAmp® PCR system 9700, Applied Biosystems, Foster City, CA, USA) 中，進行聚合酶鏈鎖反應 (polymerase chain reaction; PCR)，以增幅該菌之內轉錄間隔區 (internal transcribed spacer; ITS)。反應流程為 95℃反應 3 分鐘，35 次循環之 95℃反應 30 秒、58℃反應 30 秒、72℃反應 2 分鐘，接著 72℃反應 7 分鐘，降溫至 25℃反應 10 分鐘。經電泳確認 PCR 產物長度與完整性後，委由基龍米克斯生物科技股份有限公司 (Genomics Ltd., New Taipei, Taiwan) 進行定序，所獲得之序列再與 National Center for Biotechnology Information (NCBI) 網站上所登錄之資料庫進行 BLASTn 序列分析比對。

將菌株 TARI-PeP01 與 NCBI 資料庫中真菌序列資料進行演化樹分析。首先使用軟體 Bioedit V. 7.0.5.3 (Hall 2011) 對序列資料進行 ClustalW multiple alignment，完成後將序列資料以 MEGA XI (Tamura *et al*. 2021) 軟體進行演化樹分析，選擇最大似然法 (maximum likelihood method; ML) 方式，再以 bootstraps method 進行 1,000 次重複取樣檢驗，模式 (model) 選擇為 GTR + G + I (General Time Reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites)，呈現演化樹分析結果。此外並以 MrBayers V.3.2.7 (Ronquist & Huelsenbeck 2003) 選擇 Markov chain Monte Carlo (MCMC) 演算法進行 Bayesian concordance analysis (BCA)，同樣選擇使用 GTR + G + I 模式，MCMC 執行次數設定為 1,000,000 generations，演化樹分析以每 100 generations 取樣 1 次，檢核計算標準差 (average standard deviation of spilt frequencies) 小於 0.05。完成後捨棄前 25% 演化樹取樣結果，以 FigTree v1.4.4 軟體確認演化樹分析結果，記錄每個分支可能性 (posterior probilities; PP) 並標註於演化樹。

防治青黴菌之藥劑測試

由於目前杏鮑菇國內並無推薦藥劑，因此選用《植物保護手冊 ── 蔬菜篇》上唯一登記之香菇綠黴病菌防治參考藥劑 ── 腐絕，以及國外報導可防治柑橘青黴病 (*Penicillium digitatum*) 之撲克拉、撲克拉錳進行藥劑測試 (Zhang *et al*. 2021)。藥劑使用劑量依現行公告於《植物保護手冊 ── 蔬菜篇》中，參考使用於香菇綠黴病防治施用量作為基準進行稀釋倍數設計，分別稀釋 1,000、2,000 及 5,000 倍 (Fei & Wang 2007)。將濾紙圓片擺放於 PDA 四端點，並滴加 50 μL 稀釋之 41.8% 腐絕水懸劑、35% 撲克拉乳劑、50% 撲克拉錳可濕性粉劑及無菌水，再於 PDA 中心滴加 2 μL TARI-PeP01 之孢子懸浮液；另放置 5 mm TARI-PE01 菌絲塊於 PDA 培養基中心，同樣進行藥劑測試，以確認杏鮑菇之藥劑感受性。培養 7 日後，測量各菌落 4 個端點之菌絲半徑，並計算菌絲半徑抑制率，抑制率公式為：(a - b)/a × 100% (a 為水端點之菌絲半徑，b 為藥劑端點之菌絲半徑)。每處理共 5 皿，本試驗重複進行 3 次。

統計數據分析方法

本次將『溫度對青黴菌生長之影響測定』與『防治青黴菌之藥劑測試』試驗所得數據，以 R 4.5.1 版統計軟體進行分析，每次試驗之處理組 5 皿培養基作為試驗重複，平均後做為 1 次試驗之數據。再進行 3 次同樣試驗，並將 3 次試驗數據以單因子變異數分析法 (one-way analysis of variance; one-way ANOVA) 分析，並以杜凱確實差異檢定 (Tukey's honestly significant difference (HSD) test) 於 95% 信賴區間進行多重比較，評估各處理組間有無顯著差異，標註不同字母分群即表示具有顯著差異。

結果

青黴菌與杏鮑菇之對峙試驗

2024 年於臺中新社區發現杏鮑菇於出菇階段菌絲出現褐化並產生大量褐色菇水，嚴重影響其子實體發育與產量，且在開包菌絲表面處可見青黴病之徵兆 (圖 1A)。為瞭解杏鮑菇 TARI-PE01 與青黴菌菌株 TARI-PeP01 間的交互作用關係，將兩菌株同時置於 PDA、MEA 及 SDM 上進行對峙培養，結果顯示培養 7 日後，於 PDA 與 MEA 上可見，相較其他方向，

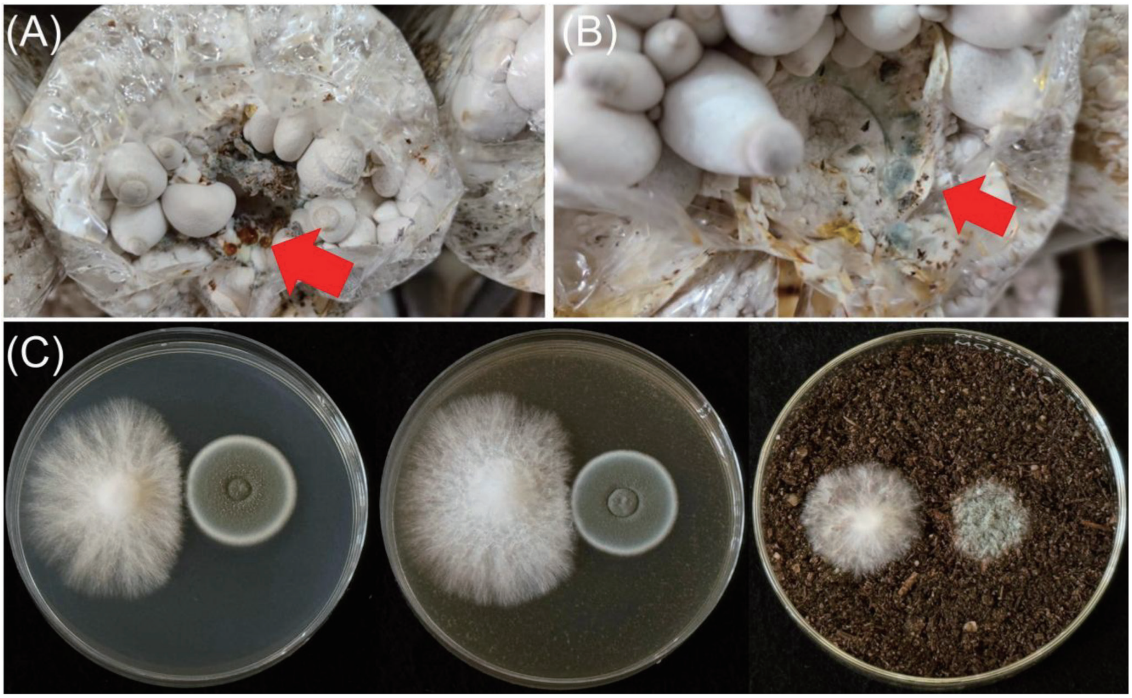

圖 1. 青黴菌 TARI-PeP01 於杏鮑菇菌絲之徵狀與杏鮑菇 TARI-PE01 之交互關係。(A) 受污染之杏鮑菇菌絲於出菇階段菌絲出現褐化，表面產生褐色菇水 (箭頭所指處)，嚴重影響其子實體產量；(B) 在開包菌絲處可見青黴菌之徵兆 (箭頭所指處)；(C) 由左至右分別為 potato dextrose agar (PDA)、malt extract agar (MEA) 及木屑培養基 (sawdust medium) 試驗結果，培養 7 日後，於 PDA 與 MEA 上之杏鮑菇菌絲生長受到抑制。

Fig. 1. Symptoms of green mold caused by TARI-PeP01 on king oyster mushroom and its interaction with *Pleurotus eryngii* strain TARI-PE01. (A) The brown mycelium and exudate (red arrows) were presented on contaminated king oyster mushroom during the development of fruiting bodies. The yield of mushroom was also affected. (B) *Penicillium* signs (red arrows) were observed on the mycelium. (C) From left to right, the images showed the dual culture results on potato dextrose agar (PDA), malt extract agar (MEA), and sawdust medium (SDM) after 7 d incubation. The mycelial growth of king oyster mushroom was affected on PDA and MEA.

接近 TARI-PeP01 菌落之杏鮑菇菌絲生長受到明顯影響；而於 SDM 則未觀察到明顯影響情形，兩者菌落亦無接觸 (圖 1B)。

青黴菌之鑑定

本研究分離之青黴菌 TARI-PeP01 鑑定係藉由顯微形態輔以分子生物學技術進行。菌落外觀形態鑑定共使用 9 種培養基進行觀察。培養 7 日後之結果顯示，TARI-PeP01 於 PDA 菌落生長大小平均為 28.6 ± 0.2 mm，菌落邊緣完整、白色，表面呈深灰綠色，皺褶不明顯，背面菌落中心部位顏色深褐色；培養於 SA 之菌落生長大小平均為 31.5 ± 0.4 mm，菌落邊緣完整，表面淺粉色，無皺褶，菌落背面無色素累積；培養於 MEA 之菌落生長大小平均為 26.8 ± 0.3 mm，菌落邊緣完整、白色，表面呈深灰綠色，皺褶不明顯，背面菌落呈深褐色；培養於 OA 之菌落生長大小平均為 24.1 ± 0.2 mm，菌落邊緣完整、白色，表面呈深灰綠色，皺褶不明顯，菌落背面不累積色素，將此培養皿密封放置 3 個月培養未觀察到菌核或有性世代；TARI-PeP01 於 YES 上菌落直徑最大，菌落大小平均為 41.3 ± 0.9 mm，表面呈灰綠色，邊緣完整、白色，皺褶明顯，背面可見黃色色素累積；DG18 屬於高滲透壓逆境培養基，常用於鑑定可在低水分環境生長的真菌，本菌株於 DG18 菌落大小平均為 30.4 ± 0.6 mm，表示此菌可在高滲透壓逆境

下生長；CREA 之菌落大小平均為 19.4 ± 0.3 mm，菌絲生長稀疏，培養基未轉為黃色，顯示此菌不產酸 (圖 2A)；CYA 之菌落大小平均為 34.1 ± 0.3 mm，菌落表面為灰綠色，邊緣完整、白色，偶有環紋狀皺褶，背面可累積少許粉色至黃色色素，CYAS 上之菌落大小平均為 33.8 ± 0.2 mm，菌落表面特徵與 CYA 接近，表面為灰綠色，邊緣白色較明顯，皺褶明顯且呈環狀或放射狀，背面不見黃色色素累積，經計算，CYA 與 CYAS 上之菌落大小比例為 0.94–1.07 (圖 2B)；在 30℃與 37℃下培養，於 CYA 未能觀察到菌絲生長 (圖 2C)。經鏡檢確認，該菌之分生孢子梗 (conidiophore) 為長度 155–203 μm，透明且多數表面無特殊突起，雙輪生 (biverticillate)，末端呈掃帚狀分支，偶有分支，其上著生 3–5 個顏色透明之梗基 (metulae)，長度 12.2–15.7 μm × 2.0–3.1 μm，每個梗基上接有 3–5 個壺狀 (ampulliform) 之產孢瓶梗 (phialide)，大小 8.1–10.3 μm × 2.2–3.6 μm，透明且末端產孢；分生孢子透明、橢圓形至圓形，大小 1.9–2.6 μm (圖 2D–H)。

將 TARI-PeP01 ITS 定序結果上傳 NCBI 資料庫 (accession number: PV523142)，並以此序列進行 BLASTn，結果顯示 TARI-PeP01 屬於青黴菌屬 (*Penicillium*) 且最相近物種為 *P. sumatraense* Svilv. (Identity: 100%)。為瞭解本菌與 Houbraken *et al*. (2011) 所鑑定同屬青黴菌屬 Section *Citrina* 物種間之親緣關係，因此選用此 section 菌株之 ITS 序列進行演化樹分析，結果顯示 TARI-PeP01 與同 section 之 Series *Sumatraensia* 真菌 (Wang *et al*. 2023) 可分在同一個演化支 (clade)，包含 *P. sumatraense*、*P. jenningsiae*、*P. cerradense*、*P. qii*、*P. vulgatum* 及 *P. rarum* (圖 3)。進一步比較親緣相近物種之形態特徵資料，結果顯示 TARI-PeP01 之菌落形態、菌落生長速度、產孢梗形式等與 *P. sumatraense* 最為接近 (表 1)，因此將所分離之菌株鑑定為 *P. sumatraense* Svilv.。

溫度對青黴菌生長之影響

為探討青黴菌於杏鮑菇栽培過程中之好發溫度，本研究測試不同培養溫度對 TARI-PeP01 在 3 種培養基 (PDA、MEA 及 SDM) 上菌落直徑之影響 (圖 4)。結果顯示，菌落直徑大小顯著受溫度影響，且 3 種培養基的反應有所不同。在 PDA 上，28℃與 24℃時之菌落直徑最大，皆超過 25 mm，但兩者間無顯著差異；20℃次之，菌落直徑約 23 mm；而 16℃之菌落直徑僅約 7 mm。在 MEA 中，菌落於 28℃之菌落直徑顯著高於其他溫度處理，而 16–24℃間，菌落仍可達 15 mm 以上。於 SDM 中，最佳生長溫度為 24–28℃，隨溫度下降，菌落直徑亦逐漸縮小；然 16℃之菌落直徑卻高於同溫條件下在 PDA 中之生長，顯示於 MEA 與 SDM 中，菌絲仍可在中低溫環境下生長。此外，在 3 種培養基中，於 32℃時均導致菌落直徑明顯下降，出現生長緩慢或未生長之情形。綜合結果可知，TARI-PeP01 菌株可於 16–28℃間生長，並以 28℃為最適生長溫度，然在 32℃下則生長受明顯抑制。

青黴菌防治藥劑之篩選

藥劑感受性試驗之結果顯示，培養 7 日後，青黴菌株 TARI-PeP01 菌落半徑不受腐絕影響，於稀釋 1,000 倍濃度下，其抑制率僅為 5.12 ± 1.71%；然其於含稀釋 2,000 倍之撲克拉與撲克拉錳之培養基上，菌落半徑剩 8.0 ± 0.41 與 6.75 ± 0.48 mm，抑制率分別為 28.98 ± 2.52% 與 32.16 ± 3.59%，與對照組 (11.25 ± 0.25 與 10.0 ± 0.71 mm) 具有顯著差異，且於其中 5,000 倍稀釋之撲克拉之培養基上，菌落半徑剩 8.5 ± 0.29 mm，同樣與對照組具有顯著差異。杏鮑菇 TARI-PE01 菌落半徑於腐絕 1,000–5,000 倍稀釋下，半徑減少 3.19–12.43%；於含稀釋 2,000 倍之撲克拉與撲克拉錳之藥劑處理下則有 22.5 ± 0.29 與 19.75 ± 0.85 mm，抑制率分別僅 8.97 ± 1.78% 與 12.27 ± 2.79%，對照組分別為生長 24.75 ± 0.63 與 22.5 ± 0.5 mm，顯示此兩種藥劑對杏鮑菇菌絲仍存在影響，但其影響較青黴菌低 (表 2)。綜上所述，顯示撲克拉與撲克拉錳對青黴菌具有明顯抑制之效力，故具有防治該菌之潛力。

討論

本研究自開包後杏鮑菇菌絲表面發現到青

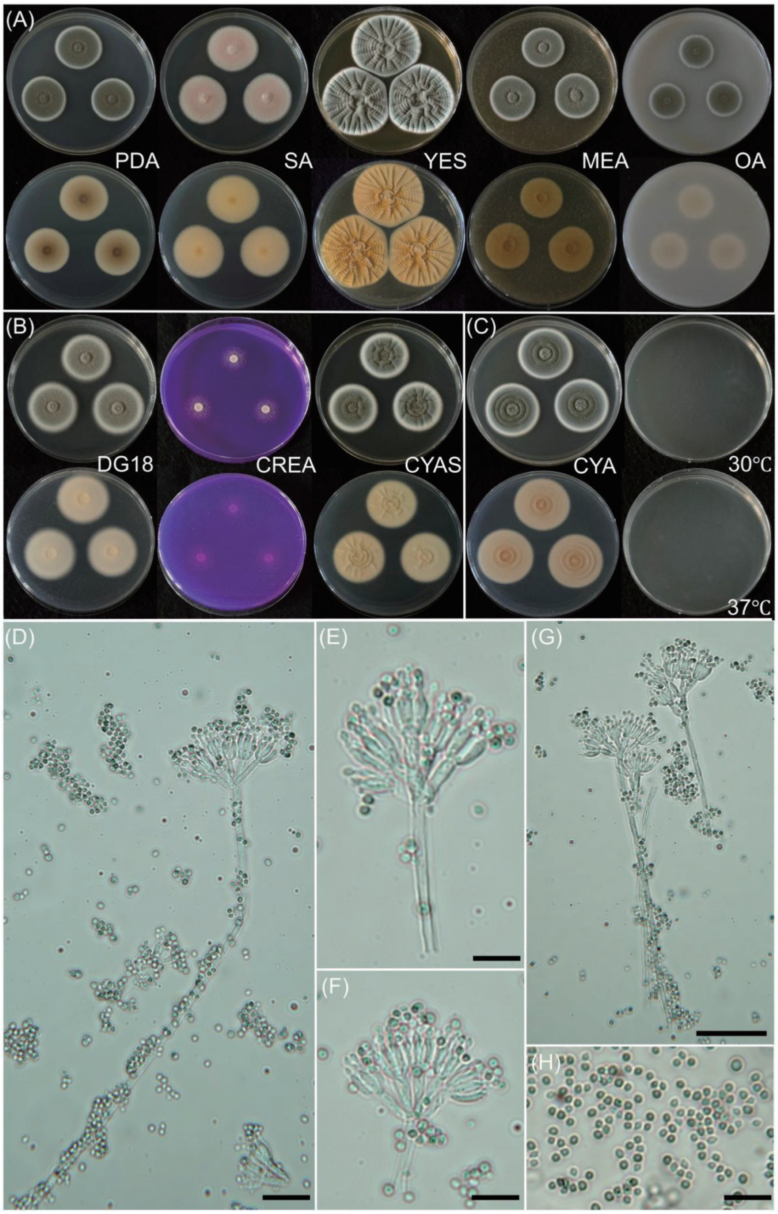

圖 2. *Penicillium sumatraense* strain TARI-PeP01 形態特徵。(A) 菌落形態特徵，由左至右分別為培養 7 日之 potato dextrose agar (PDA)、Sabouraud's agar (SA)、yeast extract sucrose agar (YES)、malt extract agar (MEA) 及 oatmeal agar (OA)；(B) 菌落形態特徵，由左至右分別為培養 7 日之 dichloran 18% glycerol agar (DG18)、creatine sucrose agar (CREA) 及 Czapek yeast autolysate agar with 5% sodium chloride (CYAS) (上行為培養基正面，下行為該反面)；(C) 於 25℃下 Czapek yeast autolysate agar (CYA) 培養 7 日後菌落形態特徵，以相同條件培養於 30℃與 37℃則無菌落生長；(D–G) 分生孢子梗；(H) 分生孢子。(Scale bar = 15 μm)

Fig. 2. The morphology of *Penicillium sumatraense* strain TARI-PeP01. (A) The colony morphology on different medium of 7 d culture under 25℃, the mediums were potato dextrose agar (PDA), Sabouraud's agar (SA), yeast extract sucrose agar (YES), malt extract agar (MEA) and oatmeal agar (OA); (B) dichloran 18% glycerol agar (DG18), creatine sucrose agar (CREA) and Czapek yeast autolysate agar with 5% sodium chloride (CYAS) (medium on first row were obverse, and second row were reverse); (C) The colony morphology on Czapek yeast autolysate agar (CYA) of 7 d culture under 25℃, 30℃ and 37℃. (D–G) Morphology of conidiophores and (H) conidia. (Scale bar = 15 μm)

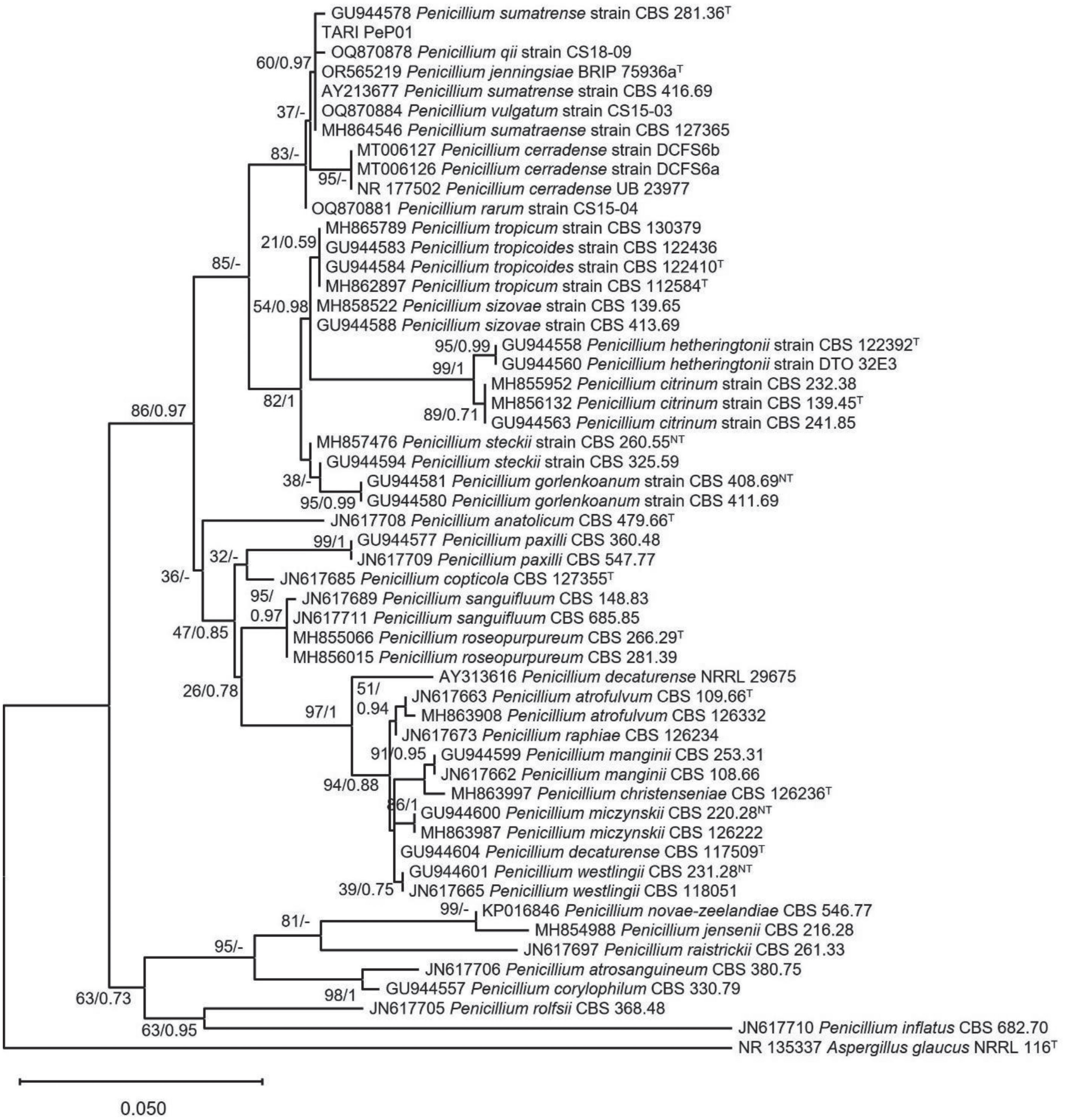

圖 3. *Penicillium sumatraense* strain TARI-PeP01 之內轉錄間隔區 (internal transcribed spacer; ITS) 親緣演化樹。以 maximun likelihood (ML) 法與 Bayesian concordance analysis (BCA) 計算各菌株親緣關係，並標註分支之 bootstrap value/posterior probabilities，比例尺為核苷酸平均替代率 (nucleotide substitution per site)。T 表示 type strain；NT 表示 neotype strain。

Fig. 3. Phylogenetic analysis of *Penicillium sumatraense* strain TARI-PeP01 based on internal transcribed spacer (ITS) sequences. Molecular phylogenetic analysis performed using the maximum likelihood (ML) method and Bayesian concordance analysis (BCA). Bootstrap value (BP)/posterior probabilities (PP) were presented at the nodes. The bar indicated the number of nucleotide substitution per site. “T” means type strain; “NT” means neotype strain.

表 1. 青黴菌屬 Section *Citrina* 相近種之形態比較。

Table 1. Morphological comparison of related species in *Penicillium* section *Citrina*.

Characteristic	*P. vulgatum*	*P. rarum*	*P. qii*	*P. jenningsiae*	*P. cerradense*	*P. sumaternse*	TARI-PeP01
Conidophore							
Size (μm)	175–425	285–515	135–275	200–315	25–240	up to 200	155–203
Branching patterns	biverticillate	terverticillate, biverticillate or monoverticillate	biverticillate, occasionally terverticillate	biverticillate	monoverticillate, biverticillate present	biverticillate	biverticillate
Metulae							
Size (μm)	12–16 × 2.5–3.0	10–15 × 2.0–3.0	12–17 × 3.0–3.5	10.5–14 × 2.5–4.5	10–20 × 2.0–3.0	12–16 × 2.0–3.0	12.2–15.7 × 2.0–3.1
Phialide							
Size (μm)	7.0–9.0 × 2.0–2.5	7.5–8.5 × 2.0–3.0	7.5–9.0 × 2.5–3.5	7.0–9.5 × 2.0–2.5	5.0–8.0 × 2.0–3.0	8.0–10 × 2.0–3.5	8.1–10.3 × 2.2–3.6
Shape	ampuliform	ampuliform	ampuliform	ampuliform	ampuliform	ampuliform	ampuliform
Sclerotia	NA[z]	NA	NA	NA	+	-	-
Cleistothecia	NA	NA	NA	NA	-	-	-
Colony diameter[y]							
YES	42–43	32–42	37–38	42–43	NA	32–42	35–45
CYA	34–35	28–36	31–32	37–38	NA	33–42	32–35
MEA	23–24	17–23	19–20	29–30	15	27–36	25–28
DG18	NA	NA	NA	NA	NA	25–34	28–34
PDA	23–25	18–21	19–21	30–32	30	NA	27–30
SA	NA	ND	NA	NA	37	NA	29–33
CYA 37℃	-	-	-	-	NA	-	-
CREA	NA	NA	NA	NA	NA	15–23	18–21
Acid production[x]	NA	NA	NA	NA	NA	-	-
Color of reverse YES	buff to yellow brown	buff with brownish cracks	buff to yellow brown	buff to yellow brown	NA	yellow	yellow
Color of reverse CYA	buff to pale brown	buff to brownish	buff to orange brown	buff	NA	beige-brown	crème to yellow
Source	Wang *et al*. 2023	Wang *et al*. 2023	Wang *et al*. 2023	Wang *et al*. 2023	Andrade *et al*. 2021	Houbraken *et al*. 2011	This study

[z] NA: no analysis.
[y] Colony diameter after 7 d incubation under 25℃. YES: yeast extract sucrose agar; CYA: Czapek yeast autolysate agar; MEA: malt extract agar; DG18: dichloran 18% glycerol agar; PDA: potato dextrose agar; SA: Sabouraud's agar; CREA: creatine sucrose agar.
[x] Acid production reaction on CREA medium.

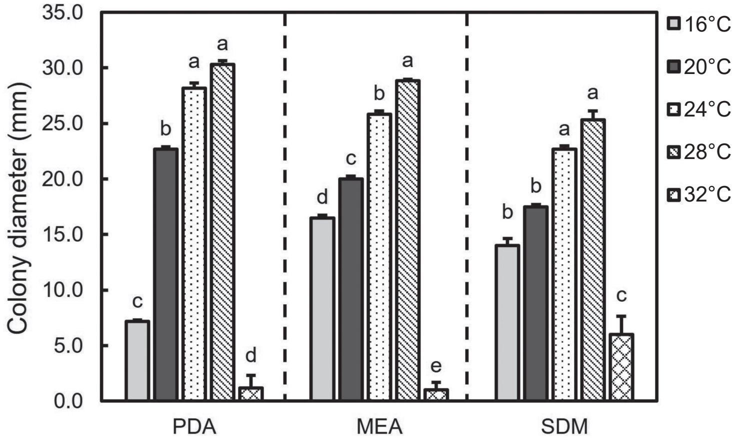

圖 4.　溫度對 TARI-PeP01 於不同培養基之生長影響。菌株 TARI-PeP01 培養於 potato dextrose agar (PDA)、malt extract agar (MEA) 及木屑培養基 (sawdust medium; SDM) 7 日之菌落直徑大小，28℃為 3 種培養基之最適生長溫度。各培養基之長條圖上方標註字母若相同表示以杜凱確實差異檢定 ($P < 0.05$) 認定不顯著。

Fig. 4.　The effect of temperatures on mycelial growth of TARI-PeP01 in different media. The colony diameter of strain TARI-PeP01 after 7 d of cultivation on potato dextrose agar (PDA), malt extract agar (MEA), and sawdust medium (SDM). The optimal growth temperature on all three media was 28℃. Values on the bars of different medium followed by the same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

表 2.　以濾紙圓盤法測試 3 種藥劑對杏鮑菇與青黴菌菌落生長半徑與抑制率之影響。

Table 2.　Using filter paper-disk method to investigate the effect of three fungicides with different dilution folds on colony radius and inhibition rate of *Pleurotus eryngii* TARI-PE01 and *Penicillium sumatraense* TARI-PeP01.

	Penicillium sumatraense strain TARI-PeP01		*Pleurotus eryngii* strain TARI-PE01	
Treatment	Colony radius (mm)	Inhibition rate (%)	Colony radius (mm)	Inhibition rate (%)
Thiabendazole				
CK[z]	14.50 ± 0.29 a	-	23.50 ± 0.96 ab[y]	-
5,000×	14.50 ± 0.29 a	0.00 ± 0.00 d	22.75 ± 0.95 abc	3.19 ± 1.08 b
2,000×	14.25 ± 0.48 a	1.79 ± 1.79 d	22.25 ± 0.75 abc	5.19 ± 1.89 b
1,000×	13.75 ± 0.25 a	5.12 ± 1.71 d	20.50 ± 0.87 bcd	12.43 ± 4.60 a
Prochloraz				
CK	11.25 ± 0.25 b	-	24.75 ± 0.63 a	-
5,000×	8.50 ± 0.29 cde	24.43 ± 2.15 c	23.50 ± 0.65 ab	4.97 ± 2.40 b
2,000×	8.00 ± 0.41 de	28.98 ± 2.52 bc	22.50 ± 0.29 abc	8.97 ± 1.78 ab
1,000×	7.00 ± 0.41 def	37.88 ± 2.62 ab	20.25 ± 0.25 bcd	17.94 ± 3.17 a
Prochloraz-Mn				
CK	10.00 ± 0.71 bc	-	22.50 ± 0.50 abc	-
5,000×	8.75 ± 0.48 cd	11.82 ± 4.61 d	21.75 ± 0.63 abcd	3.36 ± 1.13 b
2,000×	6.75 ± 0.48 ef	32.16 ± 3.59 abc	19.75 ± 0.85 cd	12.27 ± 2.79 ab
1,000×	5.75 ± 0.25 f	42.10 ± 2.00 a	18.50 ± 0.29 d	17.70 ± 1.53 a

[z] CK means water control.

[y] Data (means ± standard error) of single column with different letters are significantly different according to Tukey's honestly significant difference (HSD) test at $P < 0.05$.

黴菌之徵兆，並於樣本中發現菌絲受損與褐色菇水之產生，甚至影響其出菇產量與品質。過往之研究較少發現杏鮑菇有此種危害之發生，一般常見多是因細菌或木黴菌感染導致之病害 (Kim *et al*. 2007; González *et al*. 2009; Šašić Zorić *et al*. 2023)，而 Tian *et al*. (2017) 與 Liu *et al*. (2018) 自舞菇 (*Grifola frondosa*) 與繡球菌 (*Sparassis crispa*) 子實體分離出青黴菌，且其所造成的徵狀皆與本研究所觀察之情形相似，加上 Kim *et al*. (2020) 也指出青黴菌可抑制鴻喜菇之菌絲生長與原基體形成，因此推測青黴菌不僅可影響菇類菌絲，亦可對菇類子實體造成危害。本研究為首次發現青黴菌在杏鮑菇出菇過程中造成危害，惟過往對杏鮑菇栽培過程中青黴菌之研究多關注養菌期之危害，對於出菇期之影響研究甚少，因此值得進一步探討青黴菌株 TARI-PeP01 對杏鮑菇生長之影響，以瞭解其對杏鮑菇危害之機制。

根據 2025 年 Mycobank 資料庫統計顯示，青黴菌屬 (*Penicillium*) 中合法 (legitimate) 命名物種已累計有 1,239 個物種 (Mycobank n.d.)，因此尚需配合不同形態特徵、適溫性等生理生化特徵，並輔以多基因分子親緣分析，方能釐清相近物種的親緣關係。本文初步將分離菌株 TARI-PeP01 進行 ITS 序列分析，並將與其親緣演化樹關係相近的菌株進行顯微形態與生長特性資訊之比較 (表 1)，分離菌株之分生孢子梗屬於雙輪生 (biverticillate)，然而 *P. cerradense* 分生孢子梗形式多為單輪生 (monoverticillate)，TARI-PeP01 不僅孢子梗形式與其不符，且於 OA 上經 3 個月培養仍未能觀察到菌核產生，因此與 *P. cerradense* 具明顯之特徵區隔 (Andrade *et al*. 2021)。TARI-PeP01 分生孢子梗長度為 155–203 μm，與 *P. vulgatum* (175–425 μm) 及 *P. rarum* (285–515 μm) 不同，雖與 *P. vulgatum* 長度具有重疊範圍，然而本研究觀察到 TARI-PeP01 分生孢子梗最大僅至 203 μm，明顯與其不同 (Wang *et al*. 2023)；另比較菌落培養形態，其中於 MEA 培養 7 日後，TARI-PeP01 菌落大小為 25–28 mm，與 *P. qii* (19–20 mm) 具有差異；因此就菌落形態與顯微構造資料，TARI-PeP01 與 *P. sumatraense* 及 *P. jenningsiae* 兩菌較為相近，其中三菌於分生孢子梗長度、形式、菌落形態特徵相似，惟 *P. jenningsiae* 分生孢子梗較長 (200–315 μm)，且於 CYA 培養基的形態性狀，*P. jenningsiae* 培養 7 日後背面為淺黃色，而 *P. sumatraense* 與 TARI-PeP01 則為棕色至黃色，因此依據孢子梗形態、長度及不同培養基之菌落特徵等資訊顯示，TARI-PeP01 與 *P. sumatraense* 之形態較最為相符。雖然 Visagie *et al*. (2024) 曾藉由RNA polymerase II second largest subunit (RPB2)、calmodulin (CaM) 及 β-tubulin (BenA) 進行 MLSA，其結果將 *P. cerradense*、*P. jenningsiae* 及 *P. sumatraense* 劃分至同一演化支 (clade)。然*P. cerradense*在形態特徵上與 *P. jenningsiae* 及 *P. sumatraense* 兩者差異較大，且 Visagie *et al*. (2024) 雖有進行 MLSA 分析，但無詳細形態觀察資料，因此無法確知其所述之 *P. cerradense* 與後兩物種間形態特徵差異。而 *P. jenningsiae* 與 *P. sumatraense* 之形態特徵雖較為相近 (表 1)，但 Wang *et al*. (2023) 等人研究報告中亦缺少 *P. jenningsiae* 部分菌落培養特性資料，例如於 CREA 是否產酸之特性等，因此無法進行比較，未來若獲得 *P. jenningsiae* 菌株，可再進行試驗瞭解。此外依據 Wang *et al*. (2023) 之 MLSA 結果顯示可將兩者區別，因此未來本研究將依其 MLSA 法進一步釐清本菌之分類地位。依照目前形態鑑定結果，初步判定 TARI-PeP01 為 *P. sumatraense*。由目前之結果與青黴菌之鑑定相關研究可發現，分子證據與形態觀察在青黴菌屬 (*Penicillium*) 鑑定上皆不可或缺。

P. sumatraense 為青黴菌屬 Section *Citrina* 之真菌，該 section 之青黴菌多分離自土壤，也可自室內環境與食物上分離而來 (Pitt & Hocking 2009)，其中 *P. sumatraense* 之標準菌株 (type strain) 即自土壤分離而來。另有文獻指出該菌可能為葡萄 (*Vitis vinifera*) 之內生菌，並且可生長於軟木塞上 (Serra *et al*. 2008; Houbraken *et al*. 2011)。而本研究樣本之採集地新社區亦為臺灣葡萄重要產區，因此推測該菌之傳播來源可能與該區葡萄園有關，惟尚待

進一步驗證。此外，為釐清青黴菌在杏鮑菇生產環境中之可能發生條件，本研究針對溫度與其菌絲生長影響進行試驗，以探討其最適生長溫度範圍與潛在優勢環境，作為後續害菌管理之參考。結果顯示，本菌於 28℃下生長速度最快，然而於 MEA 與 SDM 上，菌絲仍可於 16℃低溫生長，且速度達 2 mm d^{-1}，顯示所分離之青黴菌可在杏鮑菇出菇之栽培環境 (16℃) 下，可於杏鮑菇菌絲或老菌種處持續生長並影響杏鮑菇之子實體發育，因此若未能良善管理此真菌，將導致其傳播感染至其他太空包。

進一步探討化學藥劑對此青黴菌之防治效力，本研究透過藥劑感受性試驗，以評估不同藥劑與稀釋倍數對青黴菌菌絲生長之抑制效果。結果指出，目前菇類綠黴病唯一推薦藥劑── 腐絕 (FRAC1, B1)，無法抑制 TARI-PeP01 之菌絲生長 (表 2)，因此選擇前人研究用以防治柑橘青黴菌之撲克拉 (FRAC3, G1) 與撲克拉錳 (FRAC3, G3) 進行測試。其中 5,000 倍稀釋之 35% 撲克拉 (約等於 175 mg L^{-1}) 即可顯著抑制 TARI-PeP01 生長，並僅對杏鮑菇菌絲生長造成 4.97 ± 2.40% 之抑制。雖目前初步藥劑篩選試驗所得之 TARI-PeP01 抑制率僅 24.43 ± 2.15%，然而推測因藥劑擴散導致同培養皿內之對照組較腐絕處理組相比明顯受到抑制，因此有可能導致低估藥劑之效力，故未來擬進行藥劑平板試驗確認其半數有效濃度 (median effective concentration; EC_{50})。另根據 Hatvani (2008) 試驗結果顯示，施用 250 mg L^{-1} 撲克拉時，不影響側耳屬 (*Pleurotus* spp.) 菇類子實體生長，且進一步測定施用後之子實體藥劑殘留量，發現僅能偵測到其含有 0.095 mg L^{-1} 撲克拉之殘留，遠低於衛生福利部食品藥物管理署公告蕈菜類之撲克拉殘留容許量 0.5 mg L^{-1} (https://www.mohw.gov.tw/cp-16-72181-1.html)。而杏鮑菇因同為側耳屬之菇類，雖然本研究數據顯示在目前藥劑濃度均會對杏鮑菇菌絲產生傷害，但後續仍應持續測試，以篩選出藥劑最適施用濃度，同時避免藥害發生，並分析其藥劑殘留量，以確保民眾食用之安全性。目前撲克拉雖非國內核准使用於杏鮑菇之藥劑，但由前人研究與本研究之藥劑試驗結果顯示，撲克拉對側耳屬菇類生產過程中，所發生之青黴菌具有防治潛力，而撲克拉錳則由於尚未有施用於防治杏鮑菇病害的殘留資料報告，須待更多測試才可認定是否適合施用於杏鮑菇上。本研究目前之試驗結果，未來可作為政府單位評估核准使用撲克拉作為防治藥劑之參考。

一般農民在太空包菌絲生長階段觀察到青黴菌時，即會將之丟棄，因此目前關於菇類栽培時所發生之青黴菌鑑定研究相對較少，過往也多半僅鑑定至屬之層級 (Komon-Zelazowska *et al*. 2007; Šašić Zorić *et al*. 2023)，本研究為臺灣首次自杏鮑菇菌絲分離並鑑定青黴菌，所分離出之 *P. sumatraense*，也曾被報導可危害繡球菌 (*Sparassis crispa*) 子實體 (Liu *et al*. 2018)，顯示該菌對多種菇類生長具影響，因此未來值得進一步瞭解該菌對於國內其他菇類之發生情形。此外，本研究進一步發現目前香菇綠黴病唯一推薦之防治用藥── 腐絕，對其無防治效果。依據目前試驗結果可知，撲克拉雖可作為防治青黴菌之潛在化學資材，然而考量藥劑使用所帶來之環境風險與施藥限制，未來仍應積極開發非化學農藥防治資材，如植物性萃取物或二氧化氯，並導入其他物理性防治技術，作為栽培綜合管理策略，以提升杏鮑菇生產體系之永續性與食品安全性。

引用文獻

Andrade, K. C. R., R. A. Fernandes, D. B. Pinho, M. M. de Freitas, E. X. F. Filho, A. Pessoa, ... P. O. Magalhaes. 2021. Sequencing and characterization of an L-asparaginase gene from a new species of *Penicillium* section *Citrina* isolated from Cerrado. Sci. Rep. 11:17861. doi:10.1038/s41598-021-97316-1

Arima, S. and K. Suyama. 2000. New disease, Watakabibyo showing rot symptoms in *Pleurotus eryngii* caused by *Cladobotryum varium*. Mushroom Sci. Biotech. 8:13–20. (in Japanese with English abstract) doi:10.24465/apmsb.8.1_13

Back, C. G., C. Y. Lee, G. S. Seo, and H. Y. Jung. 2012. Characterization of species of *Cladobotryum* which cause cobweb disease in edible mushrooms grown in Korea. Mycobiology 40:189–194. doi:10.5941/

MYCO.2012.40.3.189

Chen, J. T. and J. W. Huang. 2004. Identification of *Gliocladium roseum*, the causal agent of brown spot of the king oyster mushroom *Pleurotus eryngii*. Plant Pathol. Bull. 13:17–26. (in Chinese with English abstract) doi:10.6649/PPB.200403_13(1).0003

Chen, T. M., Y. S. Lu, and H. D. Shih. 2016. Current status of mushroom production in Taiwan. p.13–27. *in*: Proceedings of the Symposium on Mushroom Biotechnology. October 28, 2016. Taichung, Taiwan. Taiwan Agric. Res. Inst. Pub. No. 197, Taichung, Taiwan. (in Chinese)

Fei, W. C. and Y. C. Wang. 2007. Plant Protection Manual (Vegetables). Agricultural Chemicals Research Institute. Taichung, Taiwan. 280 pp. (in Chinese)

González, A. J., G. González-Varela, and F. J. Gea. 2009. Brown blotch caused by *Pseudomonas tolaasii* on cultivated *Pleurotus eryngii* in Spain. Plant Dis. 93:667. doi:10.1094/PDIS-93-6-0667B

Hall, T. 2011. BioEdit: An important software for molecular biology. GERF Bull. Biosci. 2:60–61.

Hatvani, L. 2008. Mushroom pathogenic *Trichoderma* species: Occurrence, biodiversity, diagnosis, and extracellular enzyme production. Doctoral Dissertation. Department of Microbiology Faculty of Science and Informatics, University of Szeged. Szeged, Hungary. 108 pp.

Houbraken, J., J. C. Frisvad, and R. A. Samson. 2011. Taxonomy of *Penicillium* section *Citrina*. Stud. Mycol. 70:53–138. doi:10.3114/sim.2011.70.02

Kim, M. K., J. S. Ryu, Y. H. Lee, and H. D. Yun. 2007. First report of *Pantoea* sp. induced soft rot disease of *Pleurotus eryngii* in Korea. Plant Dis. 91:109. doi:10.1094/PD-91-0109A

Kim, M. K., S. A. Sim, A. Y. Kim, J. H. Kwon, and Y. H. Chang. 2020. Characterization of green mold contamination caused by *Penicillium brevicompactum* in *Hypsizygus marmoreus*. Kor. J. Mycol. 48:397–405. (in Korean with English abstract) doi:10.4489/KJM.20200039

Kim, S. W., S. Kim, H. J. Lee, J. W. Park, and H. S. Ro. 2013. Isolation of fungal pathogens to an edible mushroom, *Pleurotus eryngii*, and development of specific ITS primers. Mycobiology 41:252–255. doi:10.5941/MYCO.2013.41.4.252

Komon-Zelazowska, M., J. Bissett, D. Zafari, L. Hatvani, L. Manczinger, S. Woo ... I. S. Druzhinina. 2007. Genetically closely related but phenotypically divergent *Trichoderma* species cause green mold disease in oyster mushroom farms worldwide. Appl. Environ. Microbiol. 73:7415–7426. doi:10.1128/AEM.01059-07

Lin, Y. T., Y. S. Lu, Y. T. Chang, and W. L. Deng. 2010. Bacterial decay of king oyster mushroom caused by *Burkholderia gladioli*. Plant Pathol. Bull. 19:249–254. (in Chinese with English abstract) doi:10.6649/PPB.201012_19(4).0003

Lindequist, U., T. H. J. Niedermeyer, and W. D. Jülich. 2005. The pharmacological potential of mushrooms. Evid. Based Complement Alternat. Med. 2:285–299. doi:10.1093/ecam/neh107

Liu, J., X. Li, and J. Lu. 2018. First report of blue mold on *Sparassis crispa* caused by *Penicillium sumatraense* in China. Plant Dis. 102:2380. doi:10.1094/PDIS-12-17-1908-PDN

Mycobank. n.d. Simple searching on MycoBank. https://www.mycobank.org/Simple%20names%20search (visit on 8/10/2025)

Odds, F. C. 1991. Sabouraud('s) agar. J. Med. Vet. Mycol. 29:355–359. doi:10.1080/02681219180000581

Peng, J. T., M. C. Dai, Y. F. Tsai, M. H. Chen, and J. T. Chen. 2001. Selection and breeding of king oyster mushroom. Jour. Agric. Res. China 4:43–58. doi:10.29951/JARC.200112.0005

Pitt, J. I. and A. D. Hocking. 2009. Fungi and Food Spoilage. 3rd ed. Springer. New York, NY. 388 pp. doi:10.1007/978-0-387-92207-2

Ro, H. S., E. J. Kang, J. S. Yu, T. S. Lee, C. W. Lee, and H. S. Lee. 2007. Isolation and characterization of a novel mycovirus, PeSV, in *Pleurotus eryngii* and the development of a diagnostic system for it. Biotechnol. Lett. 29:129–135. doi:10.1007/s10529-006-9206-4

Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. doi:10.1093/bioinformatics/btg180

Ruan, X. D., X. L. Zhang, and H. W. Zhang. 2008. Development and application of liquid strain in edible fungi. Liaoning Agric. Sci. 1:32–35. (in Chinese) doi:10.3969/j.issn.1002-1728.2008.01.010

Šašić Zorić, L., L. Janjušević, M. Djisalov, T. Knežić, J. Vunduk, I. Milenković, and I. Gadjanski. 2023. Molecular approaches for detection of *Trichoderma* green mold disease in edible mushroom production. Biology 12:299. doi:10.3390/biology12020299

Serra, R., S. Peterson, and A. Venâncio. 2008. Multilocus sequence identification of *Penicillium* species in cork bark during plank preparation for the manufacture of stoppers. Res. Microbiol. 159:178–186. doi:10.1016/j.resmic.2007.12.009

Tamura, K., G. Stecher, and S. Kumar. 2021. MEGA11: Molecular evolutionary genetics analysis version

11. Mol. Biol. Evol. 38:3022–3027. doi:10.1093/molbev/msab120

Tian, F. H., C. T. Li, and Y. Li. 2017. First report of *Penicillium brevicompactum* causing blue mold disease of *Grifola frondosa* in China. Plant Dis. 101:1549. doi:10.1094/PDIS-05-17-0756-PDN

Tzean, S. S., S. C. Chiu, J. L. Chen, S. H. Hseu, G. H. Lin., G. Y. Liou, ... W. H. Hsu. 1994. *Penicillium* and Related Teleomorphs From Taiwan. Food Industry Research and Development Institute. Hsinchu, Taiwan. 160 pp.

Visagie, C. M., J. Houbraken, J. C. Frisvad, S. B. Hong, C. H. W. Klaassen, G. Perrone, ... R. A. Samson. 2014. Identification and nomenclature of the genus *Penicillium*. Stud. Mycol. 78:343–371. doi:10.1016/j.simyco.2014.09.001

Visagie, C. M., N. Yilmaz, S. Kocsubé, J. C. Frisvad, V. Hubka, R. A. Samson, and J. Houbraken. 2024. A review of recently introduced *Aspergillus*, *Penicillium*, *Talaromyces* and other Eurotiales species. Stud. Mycol. 107:1–66. doi:10.3114/sim.2024.107.01

Wang, X. C., Z. K. Zhang, and W. Y. Zhuang. 2023. Species diversity of *Penicillium* in Southwest China with discovery of forty-three new species. J. Fungi 9:1150. doi:10.3390/jof9121150

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–322. *in*: PCR Protocols: A Guide to Methods and Applications. (Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White, eds.) Academic Press. San Diego, CA. 482 pp. doi:10.1016/B978-0-12-372180-8.50042-1

Yan, M. J., C. H. Jiang, and S. X. Tsai. 2002. Nutrient analysis of *Pleurotus eryngii*. Edible Fungi 24:11–12. (in Chinese)

Zhang, Y., B. Zhang, C. Luo, Y. Fu, and F. Zhu. 2021. Fungicidal actions and resistance mechanisms of prochloraz to *Penicillium digitatum*. Plant Dis. 105:408–415. doi:10.1094/PDIS-05-20-1128-RE

Identification and Fungicide Evaluation of *Penicillium sumatraense* from King Oyster Mushroom

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Abstract

Tsai, C. Y., S. S. Yu, and Y. S. Lu. 2026. Identification and fungicide evaluation of *Penicillium sumatraense* from king oyster mushroom. J. Taiwan Agric. Res. 75(2):307–321.

King oyster mushroom (*Pleurotus eryngii* (DC.) Quél.) is a commercially important cultivated mushroom in Taiwan with annual yield of 26,000 Mg and market value about 2 billion NTD. The major production areas include Taichung and Changhua. In 2024, abnormal symptoms were observed during the development of *P. eryngii* fruiting bodies in Xinshe, Taichung. An unidentified fungus caused mycelium of *P. eryngii* turn brown and produced brown exudate. The yield and quality were also affected. Signs of green mold were observed on the surface of *P. eryngii* mycelium. This study isolated a fungus (designated TARI-PeP01) from the signs and conducted dual culture assays with *P. eryngii* mycelium. Results showed that *P. eryngii* significantly reduced mycelial extension toward TARI-PeP01 on potato dextrose agar (PDA) and malt extract agar (MEA). It suggested that TARI-PeP01 had effect on mycelium of *P. eryngii*. Microscopic examination showed that conidiophores of TARI-PeP01 are 155–203 μm in length, biverticillate, and bear 3–5 metulaes per branch. Each metula carries 3–5 ampulliform phialides that produce transparent, oval to round conidia. Based on morphological characteristics and internal transcribed spacer (ITS) sequence-based phylogenetic analysis, TARI-PeP01 was identified as *Penicillium sumatraense* Svilv. Temperature assays on PDA, MEA and sawdust medium showed that TARI-PeP01 can grow at 16–28℃, with optimal growth at 28℃. However, mycelial growth was significantly inhibited at 32℃. A fungicide sensitivity assay was performed to develop potential control strategies. The results revealed that 50% prochloraz-manganese wettable powder (WP) with 2,000-fold dilution and 35% prochloraz emulsifiable concentrate (EC) with 5,000-fold dilution inhibited 32.16% and 24.43% mycelial growth of TARI-PeP01, respectively. These two fungicides with 1,000-fold dilution showed significant inhibitory effect on mycelial growth, suggesting their potential as control agents. This study is the first report of identification and control of *P. sumatraense* isolated from mycelium of king oyster mushroom. The selected fungicides provide promising options for this fungus management. Future work will focus on the physiological effects of *P. sumatraense* on *P. eryngii* and further investigation into the impact of other *Penicillium* on the mushroom industry.

Key words: *Penicillium*, King oyster mushroom, Prochloraz.

Received: August 14, 2025; Accepted: December 15, 2025.

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):323–331 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0011

研究報告

嘉義地區荔枝灌溉排程評估

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摘要

蔡耀賢、向為民、柯昱成、張哲瑋。2026。嘉義地區荔枝灌溉排程評估。台灣農業研究 75(2):323–331。

於嘉義分所荔枝試驗園土壤調查得知荔枝根系可達 120 cm 以上，而主要吸收根集中在 0–60 cm，故本研究以 60 cm 土層為基礎量測土壤基質勢含水量，計算出田間容水量及永久萎凋點分別為 212.26 mm 及 141.52 mm，以田間容水減去永久萎凋點則可得到總有效水分 70.74 mm，在乘上系數 0.6 則可估算出易有效水分 42.44 mm，最後除以灌溉效率 0.85 則可換得單次灌溉需水量為 49.93 mm。將停梢期及花芽分化期 (11 月至隔年 2月) 作物係數 (crop coefficient; Kc) 設定為 0.4、生殖生長期為 (3–6月) 設定為 0.9、營養生長期 (7–10 月) 設定為 0.7，並以嘉義分所農業氣象站之氣象資料估算參考蒸發散量 (reference crop evapotranspiration or reference evapotranspiration; ET_0)，進而估算出每一生長期之需水量，停梢期及花芽分化期在 2023 年與 2024 年分別為 150 mm 與 140 mm、生殖生長期在 2023 年與 2024 年分別為 552 mm 與 567 mm 及營養生長期在 2023 年與 2024 年分別為 421 mm 與 423 mm，得知每次灌溉有效水量及各生育時期之蒸發散量後配合每月累積雨量資料則可進行灌溉排程規劃。

關鍵詞：荔枝、灌溉排程、水分管理。

前言

在全球暖化氣候變遷下極端天氣事件發生越來越頻繁，降雨的分布不均使得果園對於灌溉需求量日益漸增，瞭解果園土壤特性與作物的需水量，對於估算灌溉需求與水資源的分配、規劃及管理至關重要 (Pereira *et al*. 2009; Dono *et al*. 2013; Wada & Bierkens 2014; Schmied *et al*. 2016)。精準灌溉可提高作物的水分利用效率與產量，但實施方面需要增進對於作物蒸散量與水分利用相關知識 (Pereira 2017)，作物於農地蒸發散損失所需的水量可視為作物需水量 (crop water requirement)，可由作物係數與潛勢蒸發量求得，而作物生產所需之灌溉需水量，是指除降雨外為滿足作物蒸散需求且不影響產量下所必須施用的水量，而需水量主要取決於作物的特性、生長階段以及環境條件，在相同的天氣條件下，不同的作物亦有不同的用水需求，不同作物在不同生育階段亦會有特定作物係數，而透過氣象資料計算參考蒸散量值，可進而估算作物在不同生育時期的消耗用水量 (Doorenbos & Pruitt 1977)。

土壤猶如海綿般布滿著孔隙而隨著土壤乾濕變化孔隙中空氣或水的比例亦會隨之改變，當降雨或灌溉後所有孔隙都充滿水時土壤處於『飽和』狀態，大孔隙中的水會透過重力自由排出，此時土壤中剩餘的水量為田間容水量 (field capacity)，剩餘的水分透過水與孔隙之間的毛細作用力或表面張力被保留在孔隙中，只能透過植物吸收或蒸發來去除，這些

投稿日期：2025 年 12 月 1 日；接受日期：2026 年 4 月 8 日。
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水可供植物吸收，而當土壤孔隙之水分降低到植物根系無法從中吸取的最低程度則為永久萎凋點 (permanent wilting poin)，而有效水分 (available water) 則可由田間容水量與永久萎凋點之間的差值所獲，從有效水分可精準估算出每次灌溉所需水量，可減少灌溉頻率與避免過度灌溉 (Veihmeyer & Hendrickson 1931; Cassel & Nielse 1986)，對水資源效率化利用，學者們為更有效節省灌溉水量與提高用水效率，會針對作物特性提出作物灌溉計畫 (Li *et al*. 2005; Wu *et al*. 2022)。

荔枝是一高產值產業，在臺灣種植面積約 1 萬公頃，年產值可達 30–40 億新臺幣，但近年來在氣候災害下導致荔枝年產量波動變大，甚至會減產至預期產量的 30% 以下 (Ghosh 2001; Teng 2005; Chang *et al*. 2025)，過去研究指出氣候變遷下，除了導致氣溫上升還可能影響降雨型態的改變，這都可能直接造成作物產量與品質的降低 (Solomon *et al*. 2009; Kumar 2014; Huang *et al*. 2019; Hwang *et al*. 2024)，為因應氣候變遷下所造成水資源短缺的挑戰，必須從瞭解作物需水特性、創新灌溉設備與技術、調整種植模式、優化灌溉系統等著手來減少灌溉用水量從而提高用水效率 (Si *et al*. 2020; Jabeen *et al*. 2022)。

材料與方法

本試驗地點為農試所嘉義分所荔枝試驗園，先於試驗園內進行土壤剖面挖掘，並依各土層性質分成 0–16、16–30、30–55 及 55–75 cm，以 100 mL 容積不銹鋼管分層採取不經擾動 (undisturbed soil sample) 土壤樣品，同時亦採各層之分散土壤樣品 (disturbed soil sample)，土壤樣品採集後依照 Soilmoisture Equipment Corp. 出版的操作說明書，實施高壓壓力鍋與低壓壓力鍋之保水含水量測定，高壓壓力分別為 80、100、300、500、800、1,000 及 1,500 kPa，取用過 2 mm 篩網的散土壤樣品；低壓壓力分別為 0、10、33 及 50 kPa，取用未受干擾的土壤樣本，將飽和的土壤樣本置於壓力鍋中的陶瓷板上蓋緊壓力鍋的蓋子，逐漸調升壓力至試驗所需，平衡後的土壤樣本以 105℃下乾燥至恆重，計算出水分含量。

至農業氣象觀測網監測系統 (https://agr.cwa.gov.tw/) 選擇農試所嘉義分所氣象站 (G2L020)，下載最低溫、最高溫、濕度、風速、日照時數降雨量等，並估算作物需水量。

作物蒸發散量 (ETcrop)，可以經由 ET_0 得到，其關係式為：

ETcrop = Kc × ET_0

ETcrop：作物蒸發散量或作物需水量；

ET_0：蒸發散量參考值；

Kc：作物係數；

ET_0 以 Penman-Monteith 公式計算。

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma\dfrac{900}{T + 273}u_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)}$$

其中：

ET_0：參考蒸發散量 (mm day^{-1})；

R_n：全天空日射量 (MJ m^{-2} day^{-1})；

G：地表熱通量 (MJ m^{-2} day^{-1})；

T：日平均氣溫 (℃)；

u_2：離地表 2 m 之風速 (m s^{-1})；

e_s：飽和蒸汽壓 (kPa)；

e_a：實測蒸氣壓 (kPa)；

$e_s - e_a$：和蒸汽壓差 (kPa)；

Δ 水蒸氣飽和曲線對溫度作圖之斜率 (kPa $℃^{-1}$)；

r：乾溼度常數 (kPa $℃^{-1}$)。

田間容水量 (field capacity) 與永久萎凋點估算，以土層深度分別乘以 10 kPa 及 1,500 kPa 之土壤含水量並進行累加，而總有效水分含量則是以田間容水量減去永久萎凋點，容易有效水分含量 (readily available moisture) 是總有效水分含量乘以 P 的乘積，並以此為依據估算灌溉需水量 (Allen *et al*. 1998; Zotarelli *et al*. 2010)。

d (mm) = (Sa × P)；

Sa：總有效水分含量；mm/m；

P：是允許消耗的有效水分；分數；

D：根深 (m)。

結果與討論

作物係數由作物品種與栽培方式決定，亦隨作物的生育階段而變動。荔枝的作物係數，依照南非的量測資料，在 0.4–1.2 之間，並沒有季節性趨勢，參考學者專家所發表 Kc 值，本研究將 Kc 值設定為停梢期與花芽分化期為 0.4 (11 月至隔年 2 月)、生殖生長期為 0.9 (3–6 月)、營養生長期 0.7 (7–10 月) (Carr & Menzel 2014; Pereira *et al*. 2024)，透過設定不同生長期的作物係數 (Kc) 與嘉義農業試驗分所 2023 年與 2024 年所測量的氣象資料可計算出各生育期的作物需水量 (ETcrop) 如表 1，2023 年與 2024 年合計年度作物需水量分別為 1,120 mm 與 1,130 mm，所估算出荔枝年度需水量與 Mostert (2012) 研究指出荔枝年需水量介於 900–1,100 mm 之間，日需水量介於 1–5 mm 之間結果相符，2023 年與 2024 年合計年累積雨量分別為 1,524.5 mm 與 2,256.0 mm，遠高於荔枝年度需水量，11 月至隔年 2 月為停梢期與花芽分化期，在 2023 年與 2024 年作物需水量分別為 149 mm 與 140 mm，而降雨量分別為 26.0 mm 與 120.0 mm；3–6 月為生殖生長期，2023 年與 2024 年作物需水量分別為 552 mm 與 566 mm，而降雨量分別為 408.5 mm 與 189.0 mm；7–10 月為營養生長期，2023 年與 2024 年作物需水量分別 419 mm 與 424 mm，而降雨量分別為 1,090.0 mm 與 1,947.0 mm (表 1)。

雖然臺灣年均降雨量高於荔枝的年度需水量，但降雨的分布存在季節間差異且荔枝各生長階段亦有不同作物需水量，將造成作物缺水必須仰賴適度灌溉，而精準灌溉水量可從土壤有效水分含量進行估算，於嘉義試驗分所荔枝試驗田進行土壤剖面挖掘，土壤剖面質地顯示表土 0–16 cm 質地為壤土，剖面 16 cm 以下之土壤質地均屬粘重的黏土類土壤，總體密度在 1.2–1.5 g/cm^3，土系包括陳厝寮系與盧厝系等土系與 Chen *et al*. (2009) 研究站土壤特性一致。之後根據每一土層性質以 0–16、16–30、30–55 及 55–75 cm 進行分層採樣；而觀察荔枝根系發現在土壤剖面下 120 cm 深處仍可觀察到荔枝根系存在，Menzel *et al*. (1990) 的調查顯示荔枝 80% 的吸收根 (feeder roots) 局限在土表面下 0–20 cm 至 0–60 cm 之間，本次土壤剖面調查亦顯示主要根系集中於 60 cm 以上土層 (圖 1)，因此可將荔枝的有效根深設定為 60 cm。土壤有效含水可用來量化土壤能夠儲存並供給植物根系吸收利用的水分範圍，亦可用來估算每次灌溉水量已達精準灌溉，以壓力鍋之保水含水量測定在壓力平衡下的土壤水分含量，量測結果如表 2 在 10 kPa 下 0–16、16–30、30–55 及 55–75 cm 各層土壤水分含量分別為 34.7%、29.7%、37.7% 及 41.7%，1,500 kPa 下 0–16、16–30、30–55 及 55–75 cm 各層土壤水分含量分別為 11.9%、26.8%、28.5% 及 27.8% (表 2)，田間容水量與永久萎凋點以各土層深度分別乘以 10 kPa 與 1,500 kPa 之土壤含水量並進行累加，計算出田間容水量與永久萎凋點分別為 212.26 mm 與 141.52 mm，總有效水分含量則是以田間容水量減去永久萎凋點為 70.74 mm，而容易有效水分含量或允許消耗的有效水分含量 (permissible deficit or depletion of soil available water) 為易於被植物吸收沒有任何導致產量減損的壓力的部分，一般占總有效水分含量的 20–70%，可以是總有效水分含量乘以 P 的乘積估算，深根大田作物與成熟的果樹為 0.40–0.60，所估算容易有效水分含量 42.44 mm，最後將其水量除以灌溉效率，灌溉效率以滴灌 0.85 進行計算 (Howell 2003)，1 次灌溉需水量約為 49.93 mm，可在不同時期進行灌溉需求評估，當作物蒸發散量達 42.44 mm 時，即可進行單次約為 50 mm 灌溉，相當於 500 Mg ha^{-1}。在無法量測土壤有效含水量或不同田區的狀況下，可依據 Morris (2023) 灌溉指引查表可得知上層 (0–16 cm) 壤土土壤有效水分含量為 2 (in/ft)，底層 (16–75 cm) 坋質黏壤土土壤有效水分含量為 2.4 (in/ft)，土壤有效水量估計值為 114.7 mm，容易有效水分含量為 68.8 mm，一次灌溉需水量約為 81 mm，在無法量測土壤有效含水量可依據灌溉指引估算出灌溉需水量，但有高估之趨勢。

荔枝的灌溉排程可由降雨量、灌溉水量及作物生長階段需水量與生理特性進行評估，在 11 月至隔年 2 月為停梢期與花芽分化期，此

表 1. 荔枝生育期之作物蒸發散量、月累積雨量及建議灌溉量。

Table 1. Crop evapotranspiration, monthly accumulated precipitation, and recommended irrigation during the litchi growth period.

Growth stage	2023 Crop evapotranspiration (mm)	2023 Monthly accumulated precipitation (mm)	2024 Crop evapotranspiration (mm)	2024 Monthly accumulated precipitation (mm)	Recommended irrigation (mm)
Terminal bud period and flower bud differentiation period	149	26.0	140	120.0	After flower bud differentiation 50 mm/mo
November	40	0.0	37	63.5	
December	34	15.5	31	21.5	
January	35	10.5	34	2.0	
February	40	0.0	38	33.0	
Reproductive growth period	552	408.5	566	189.0	March to May 50 mm/14 d June 50 mm/10 d
March	122	8.0	121	5.5	
April	129	64.0	144	128.0	
May	150	127.5	144	45.5	
June	151	209.0	157	10.0	
Vegetative growth period	419	1,090.0	424	1,947.0	30 mm/7 d Promoting flush 50 mm/once
July	123	290.5	120	1,282.5	
August	117	193.0	117	256.0	
September	93	562.5	100	331.5	
October	86	44.0	87	77.0	
Total	1,120	1,524.5	1,130	2,256.0	

圖 1. 嘉義分所荔枝試驗園土壤剖面。

Fig. 1. Soil profile of the litchi experimental orchard at the Chiayi Agricultural Experiment Branch.

時期亦為花芽分化的重要階段，對產量影響至關重要，而荔枝開花亦需仰賴充足的低溫，誘導後續一系列開花基因的表現與調控 (Bernier 1988; Shen *et al*. 2016)。Ding *et al*. (2015) 研究指出荔枝成花關鍵基因 *FLOWERING LOCUS T* (*LcFT1*) 受環境低溫調節表現，使得早熟品種「三月紅」 (‘Sanyuehong’) 與晚熟品種「馬貴荔」 (‘Maguili’) 分別在 11 月初與 1 月進入花芽誘導與分化，雖然開花前乾旱逆境有助於荔枝停梢管理與花芽分化，但在嚴重缺水下即使滿足低溫條件亦難以產生花芽 (Carr & Menzel 2014)，因此可待花芽分化穩定後，可間隔約一個月進行一次 50 mm 灌溉， 3–6 月為生殖生長期此時期需水量為最高，這與荔枝在開花結果時對水分的需求增加的生理特性相符，3–5 月為盛花期與果實發育初期每日蒸發散損失約 3 mm，其容易有效水分含量為 42.44 mm，可供作物 14 日的蒸發散需求。如連續 14 日無降雨，則建議行 1 次 50 mm 灌溉。

表 2. 土壤不同基質勢下之體積含水量 (單位：v/v%)。

Table 2. Volumetric water content at different soil matric potentials (unit: v/v%).

Soil layer (cm)	Soil matric potential (kPa)										
	0	10	33	50	80	100	300	500	800	1,000	1,500
0–16	43.6 ± 1.6	34.7 ± 1.8	32.9 ± 1.5	32.4 ± 0.9	17.6 ± 1.1	23.2 ± 1.5	15.9 ± 0.8	9.1 ± 1.0	19.8 ± 0.2	11.3 ± 1.1	11.9 ± 0.6
16–30	35.2 ± 1.2	29.7 ± 1.0	26.0 ± 0.8	24.1 ± 0.9	30.6 ± 2.0	29.3 ± 1.6	24.0 ± 0.3	21.1 ± 0.9	23.9 ± 0.6	22.9 ± 0.5	26.8 ± 0.3
30–55	40.2 ± 1.5	37.7 ± 1.1	36.4 ± 0.7	35.9 ± 1.0	33.1 ± 1.8	30.8 ± 0.8	27.3 ± 0.2	24.7 ± 0.8	26.9 ± 0.5	25.7 ± 0.7	28.5 ± 1.1
55–75	45.5 ± 0.8	41.7 ± 1.6	40.1 ± 0.7	39.7 ± 1.5	32.1 ± 1.5	29.9 ± 0.9	26.8 ± 0.2	23.7 ± 1.0	26.2 ± 1.2	25.3 ± 0.9	27.8 ± 1.4

6 月進入果實成熟採後及修剪期每日蒸發散損失約 4 mm，可供作物 10 日的蒸發散需求，如連續 10 日無降雨則可進行 1 次 50 mm 灌溉，7–9 月為營養生長期，此時期每日蒸發散損失約 3–5 mm，可供作物 8–14 日的蒸發散需求，鑒於本階段處於雨季且果實業已採收完畢，灌溉以補充灌溉的方式施行，可在連續 7 日無降雨時少量灌溉 1 次 30 mm，至晚秋進行催梢時充分灌溉 1 次 50 mm (圖 2–4、表 1)。

臺灣橫跨熱帶與亞熱帶氣候區，得天獨厚的地理位置帶來豐沛年降雨量，極適合荔枝栽培，然降雨分布高度集中於春雨季 (2–4月)、梅雨季 (5–6月) 及颱風季 (7–9月)，且年際間差異極大，常遭遇春雨不至、空梅或無颱風過境等極端氣候挑戰；以 2023 年降雨分布來看，春雨期 2–4 月分別為 0、8 及 64 mm，梅雨期 5–6 月為 127.5 mm 與 209 mm，颱風季 7–9 月

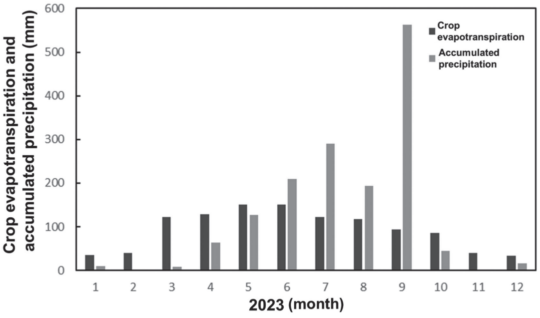

圖 2. 2023 年作物蒸發散量與月累積雨量。

Fig. 2. Crop evapotranspiration and monthly accumulated rainfall for 2023.

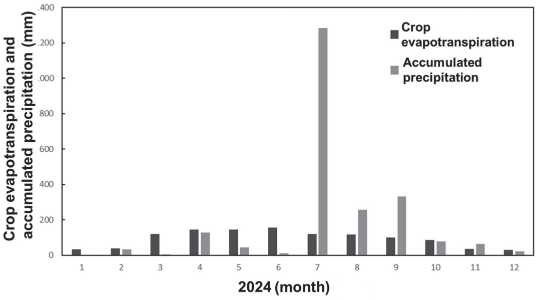

圖 3. 2024 年作物蒸發散量與月累積雨量。

Fig. 3. Crop evapotranspiration and monthly accumulated rainfall for 2024.

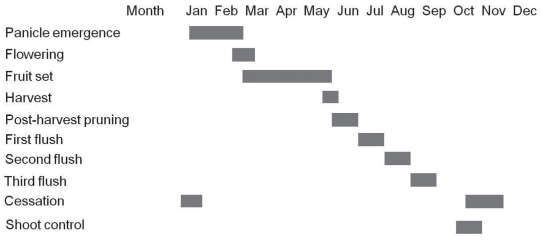

圖 4. 荔枝「黑葉」生產曆與物候期。

Fig. 4. Production calendar and phenological phases of 'Hei Ye' litchi.

則為 290.5、117 及 562.5 mm，而 10 月至隔年 1 月分別為 44、0、15.5 及 10.5 mm；對比 2024 年，春雨期 2–4 月為 33、5.5 及 128 mm，梅雨期 5–6 月僅 45.5 mm 與 10 mm，颱風季 7–9 月則高達 1,282.5、256 及 331.5 mm，而 10 月至隔年 1 月分別為 77、63.5、21.5 及 2 mm。基本上，10 月後若無熱帶氣旋活動，氣候將趨於乾旱，但仍可能有秋颱與東北季風交會造成的劇烈降雨，面對此種極端且不穩定的降雨模式，果園應建立完善的因應方案，如建置蓄水設施並搭配滴灌系統等以提高水分利用效率。在停梢期與花芽分化期 (11月至隔年 2月)，2023 年因降雨量嚴重不足，應於花穗分化穩定後進行一次 50 mm 的灌溉，而 2024 年降雨較充足，可依植株狀況減少灌溉量；在生殖生長期 (3–6月)，2023 年除 3 月與 4 月需每 14 日進行一次灌溉外，其餘月分降雨尚屬充足，2024 年則除 4 月雨水較豐沛外，其餘 3、5 及 6 月均需每 14 日灌溉一次，若遇極端乾旱導致儲水不足，則應採取關鍵期灌溉策略，優先供應果實發育所需。至於營養生長期 (7–9月)，2023 年與 2024 年降雨皆極為充沛。由於臺灣降雨分布年度差異顯著，難以將灌溉管理標準化，應透過記錄降雨量、評估作物需水量，並結合蓄水設施與節水方案進行動態決策，方能達成荔枝之精準灌溉與抗旱管理。

結論

本研究針對臺灣荔枝精準灌進行評估，估算 2024 年與 2025 年度總需水量約為 1,122–1,131 mm，荔枝不同生育期之作物需水量隨

生長階段變動，其中以 3–6 月生殖生長期之 Kc 值 0.9 為需水最高峰，11 月至隔年 2 月停梢期 Kc 值 0.4 為最低；經由土壤剖面調查與壓力鍋測定，確認荔枝有效根深為 60 cm，並計算出容易有效水分含量為 42.44 mm，並以此作為起灌點之科學依據，即當累積蒸發散量達此閾值且無降雨時，應單次灌溉約 50 mm 以維持植株生理需求，可應用於臺灣降雨季節分布極端不均且年差異巨大的挑戰，灌溉管理應捨棄固定排程，改採動態決策模式，於花芽分化期適度控水並在穩定後補充灌溉，於生殖生長期嚴密監控降雨缺口以防落果，於營養生長期則善用雨水並在晚秋催梢時充分灌溉，透過整合氣象觀測、土壤物理特性及作物生理特性，建立結合蓄水與節水滴灌之精準模式，方能於極端氣候下有效提升水分利用效率並穩定產量。

引用文獻

Allen, R. G., L. S. Pereira, D. Raes, and M. Smith. 1998. Crop Evapotranspiration- Guidelines for Computing Crop Water Requirements. FAO Irrig. Drain. Pap. No. 56. Rome, Italy. 300 pp.

Bernier, G. 1988. The control of floral evocation and morphogenesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39:175–219. doi:10.1146/annurev.pp.39.060188.001135

Carr, M. K. V. and C. M. Menzel. 2014. The water relations and irrigation requirements of lychee (*Litchi chinensis* Sonn.): A review. Exp. Agric. 50:481–497. doi:10.1017/S0014479713000653

Cassel, D. K. and D. R. Nielsen. 1986. Field capacity and available water capacity. p.901–926. *in*: Methods of Soil Analysis, Part 1: Physical and Mineralogical Methods. (Klute, A., ed.). American Society of Agronomy and Soil Science Society of America. Madison, WI. 1188 pp. doi:10.2136/sssabookser5.1.2ed.c36

Chang, J. Y., L. Tang, C. C. Chen, Y. T. Zhang, and C. L. Chen. 2025. Prediction of inflorescence emergence in "Yu Her Pau" litchi under climate change using an optimised model. Food Energy Secur. 14:e70032. doi:10.1002/fes3.70032

Chen, C. L., B. H. Chen, S. N. Huang, H. B. King, W. C. Sun, H. Y. Guo, ... Z. S. Chen. 2009. Establishment and perspectives of a long term ecological research in agricultural ecosystem in subtropics. Crop Environ. Bioinform. 6:233–246. (in Chinese with English abstract) doi:10.30061/CEB.200912.0004

Ding, F., S. Zhang, H. Chen, Z. Su, R. Zhang, Q. Xiao, and H. Li. 2015. Promoter difference of *LcFT1* is a leading cause of natural variation of flowering timing in different litchi cultivars (*Litchi chinensis* Sonn.). Plant Sci. 241:128–137. doi:10.1016/j.plantsci.2015.10.004

Dono, G., R. Cortignani, L. Doro, L. Giraldo, L. Ledda, M. Pasqui, and P. P. Roggero. 2013. An integrated assessment of the impacts of changing climate variability on agricultural productivity and profitability in an irrigated Mediterranean catchment. Water Resour. Manage. 27:3607–3622. doi:10.1007/s11269-013-0367-3

Doorenbos, J. and W. O. Pruitt. 1977. Crop Water Requirements. FAO Irrig. Drain. Pap. No. 24. Rome, Italy. 154 pp.

Ghosh, S. P. 2001. World trade in Litchi: Past, present and future. Acta Hortic. 588:23–30. doi:10.17660/ActaHortic.2001.558.1

Howell, T. A. 2003. Irrigation efficiency. p.467–472. *in*: Encyclopedia of Water Science. (Stewart, B. A. and T. A. Howell, eds.) Marcel Dekker, New York, NY. 1076 pp.

Huang, F., T. Du, S. Wang, X. Mei, D. Gong, Y. Chen, and S. Kang. 2019. Current situation and future security of agricultural water resources in North China. Strateg. Study CAE 21(5):28–37. (in Chinese with English abstract) doi:10.15302/J-SSCAE-2019.05.024

Hwang, Y. W., Y. H. Hsu, and Y. M. Chen. 2024. Impact of flowering temperature on litchi yield under climate change: A case study in Taiwan. Clim. Serv. 34:100483. doi:10.1016/j.cliser.2024.100483

Jabeen, M., S. R. Ahmed, and. M. Ahmed. 2022. Enhancing water use efficiency and grain yield of wheat by optimizing irrigation supply in arid and semi-arid regions of Pakistan. Saudi J. Biol. Sci. 29:878–885. doi:10.1016/j.sjbs.2021.10.018

Kumar, R. 2014. Effect of climate change and climate variable conditions on Litchi (*Litchi chinensis* Sonn.) productivity and quality. Acta Hortic. 1029:145–154. doi:10.17660/ActaHortic.2014.1029.16

Li, J., S. Inanaga, Z. Li, and A. E. Eneji. 2005. Optimizing irrigation scheduling for winter wheat in the North China Plain. Agric. Water Manag. 76:8–23. doi:10.1016/j.agwat.2005.01.006

Menzel, C. M., R. L. Aitken, A. W. Dowling, and D. R. Simpson. 1990. Root distribution of lychee trees growing in acid soils of subtropical Queensland. Aust. J. Exp. Agric. 30:699–705.

Morris, M. 2023. The Irrigator's Pocket Guide. National Center for Appropriate Technology. Butte, MT. 21 pp.

Mostert, P. G. 2012. Irrigation of litchis in South Africa- An overview. Acta Hortic. 1029:177–182. doi:10.17660/ActaHortic.2014.1029.20

Pereira, L. S. 2017. Water, agriculture and food: Challenges and issues. Water Resour. Manag. 31:2985–2999. doi:10.1007/s11269-017-1664-z

Pereira, L. S., I. Cordery, and I. Iacovides. 2009. Coping with Water Scarcity: Addressing the Challenges. Springer, Dordrecht, The Netherlands. 382 pp. doi:10.1007/978-1-4020-9579-5

Pereira, L. S., P. Paredes, C. M. Oliveira, F. Montoya, R. López-Urrea, and M. Salman. 2024. Single and basal crop coefficients for estimation of water use of tree and vine woody crops with consideration of fraction of ground cover, height, and training system for Mediterranean and warm temperate fruit and leaf crops. Irrig. Sci. 42:1019–1058. doi:10.1007/s00271-023-00901-7

Schmied, H. M., L. Adam, S. Eisner, G. Fink, M. Flörke, H. Kim, ... P. Döll. 2016. Variations of global and continental water balance components as impacted by climate forcing uncertainty and human water use. Hydrol. Earth Syst. Sci. 20:2877–2898. doi:10.5194/hess-20-2877-2016

Shen, J., Q. Xiao, H. Qiu, C. Chen, and H. Chen. 2016. Integrative effect of drought and low temperature on litchi (*Litchi chinensis* Sonn.) floral initiation revealed by dynamic genome-wide transcriptome analysis. Sci. Rep. 6:32005. doi:10.1038/srep32005

Si. Z., M. Zain, F. Mehmood, G. Wang, Y. Gao, and A. Duan. 2020. Effects of nitrogen application rate and irrigation regime on growth, yield, and water-nitrogen use efficiency of drip-irrigated winter wheat in the North China Plain. Agric. Water Manag. 231:106002. doi:10.1016/j.agwat.2020.106002

Solomon, S., G. K. Plattner, R. Knutti, and P. Friedlingstein. 2009. Irreversible climate change due to carbon dioxide emissions. Proc. Natl. Acad. Sci. U.S.A. 106:1704–1709. doi:10.1073/pnas.0812721106

Teng, Y. S. 2005. Lychee production in Taiwan. Acta Hortic. 665:43–46. doi:10.17660/ActaHortic.2005.665.3

Veihmeyer, F. J. and A. H. Hendrickson. 1931. The moisture equivalent as a measure of the field capacity of soils. Soil Sci. 32:181–194. doi:10.1097/00010694-193109000-00003

Wada, Y., and M. F. P. Bierkens. 2014. Sustainability of global water use: Past reconstruction and future projections. Environ. Res. Lett. 9:104003. doi:10.1088/1748-9326/9/10/104003

Wu, R. S., H. C. Wu, and Y. F. Ma. 2022. On estimation of irrigation water consumption in Taiwan. J. Taiwan Agric. Eng. 68(4):18–34. (in Chinese with English abstract) doi:10.29974/JTAE.202212_68(4).0001

Zotarelli, L., M. D. Dukes, C. C. Romero, K. W. Migliaccio, and K. T. Morgan. 2010. Step by step calculation of the Penman-Monteith evapotranspiration (FAO-56 method). EDIS 2010:AE459. doi:10.32473/edis-ae459-2010

Litchi Irrigation Scheduling Assessment in Chiayi Area

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Abstract

Tsai, Y. S., W. M. Siang, Y. C. Ko, and J. W. Chang. 2026. Litchi irrigation scheduling assessment in Chiayi area. J. Taiwan Agric. Res. 75(2):323–331.

Based on a soil survey of the litchi experimental orchard at the Chiayi Agricultural Experiment Branch, it was observed that the root system of lychee trees can reach depths of over 120 cm, while the primary absorbing roots are concentrated in the 0–60 cm soil layer; consequently, this study used the 60 cm soil profile as the basis for measuring soil matric potential and water content, calculating the field capacity and permanent wilting point to be 212.26 mm and 141.52 mm, respectively, which determined the total available water to be 70.74 mm, and by applying a depletion coefficient of 0.6, the readily available water was estimated at 42.44 mm, which, when divided by an irrigation efficiency of 0.85, resulted in a gross irrigation requirement of 49.93 mm per application. Furthermore, the crop coefficients were set at 0.4 for the terminal bud period and flower bud differentiation period (November to February), 0.9 for the reproductive growth period (March to June), and 0.7 for the vegetative growth period (July to October), and by using meteorological data from the Chiayi Agricultural Experiment Branch to estimate the reference evapotranspiration, the water requirements for each growth stage were determined to be 150 mm and 140 mm for the terminal bud period and flower bud differentiation period, 552 mm and 567 mm for the reproductive growth period, and 421 mm and 423 mm for the vegetative growth period in 2023 and 2024 respectively, allowing for the formulation of an irrigation schedule based on the effective irrigation water per application, the evapotranspiration of each growth period, and monthly cumulative rainfall data.

Key words: Litchi, Irrigation schedule, Water management.

Received: December 1, 2025; Accepted: April 8, 2026.

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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):333–349 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0012

Research Article

Moderate Roasting Improves Bioactivity and Stability of Sacha Inchi (*Plukenetia volubilis* L.) Oil Without Altering Its Fatty Acid Composition

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Abstract

Lee, Y. L., T. H. Hung, W. T. Liu, S. Y. Lin, Y. H. Ho, and Y. F. Lee. 2026. Moderate roasting improves bioactivity and stability of Sacha Inchi (*Plukenetia volubilis* L.) oil without altering its fatty acid composition. J. Taiwan Agric. Res. 75(2):333–349.

Sacha Inchi oil (*Plukenetia volubilis* L.), rich in ω-3 fatty acids and vitamin E, has attracted growing global interest for its potential health benefits. However, its low oxidative stability remains a key limitation to commercial application. This study investigated the effects of moderate thermal pre-treatment (roasting) on the chemical composition, oxidative stability, and functional bioactivity of Sacha Inchi oil, comparing oils extracted from roasted and unroasted seeds. Ten seed samples were collected from six regions in Taiwan: Taichung, Changhua, Nantou, Chiayi, Tainan, and Hualien. After dehulling, the seeds were mechanically pressed without intentional heating to obtain oil. Analytical parameters included acid value (AV), peroxide value (POV), oil stability index (OSI, at 130℃), vitamin E content, fatty acid profile, phospholipid composition (via thin-layer chromatography), total phenolic content, reducing power, and anti-inflammatory activity assessed using a cell-based model. Results showed that roasting did not alter the fatty acid profile or vitamin E content, but significantly improved oxidative stability (higher OSI) and promoted the accumulation of bioactive compounds such as phospholipids (ex. phosphatidylethanolamine; PE) and total phenolics, along with stronger reducing power. Both roasted and unroasted oils exhibited anti-inflammatory activity, with roasted oil showing a slightly greater suppression of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated macrophages. In summary, controlled roasting effectively enhances the oxidative stability, bioactive composition, and physiological functionality of Sacha Inchi oil while preserving its core nutritional profile (rich in ω-3 fatty acids and vitamin E). These findings support roasting as a feasible strategy to enhance the stability and bioactivity of Sacha Inchi oil for food and nutraceutical applications.

Key words: Sacha Inchi oil, Roasting, Oxidative stability, Functional bioactivity, Anti-inflammatory activity.

Received: October 14, 2025; Accepted: December 5, 2025.
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INTRODUCTION

Sacha Inchi (*Plukenetia volubilis* L.) is a perennial oilseed vine native to the eastern slopes of the Andes, particularly in Peruvian regions such as Cusco, Junín, and Pasco, where it thrives at altitudes of 1,600–2,100 m. Although its natural distribution ranges from the Lesser Antilles to northern South America, the Andean populations display the greatest morphological diversity and have a long history of traditional use by indigenous communities (Bussmann *et al*. 2013). In recent years, Sacha Inchi oil has attracted international attention due to its high content of polyunsaturated fatty acids (PUFAs), especially α-linolenic acid (ALA), as well as other bioactive compounds such as tocopherols, phytosterols, and phenolics (Mhd Rodzi & Lee 2022; Mhd Rodzi *et al*. 2025). Clinical and preclinical studies have demonstrated its potential benefits in regulating blood lipids, reducing inflammation, and supporting cardiovascular health (Mhd Rodzi *et al*. 2025). Recognizing its nutritional value, Peru has actively promoted Sacha Inchi oil as a novel food and proposed its inclusion in the Codex Standard for Named Vegetable Oils (CAC 2024).

Despite these health-promoting properties, Sacha Inchi oil is traditionally extracted via cold pressing without heat treatment, based on the prevailing belief that PUFAs are highly susceptible to thermal degradation, as noted in Peru's submission to CAC (2021). This perception has led to the avoidance of roasting or any form of pre-heating during processing. However, studies on other oilseeds, such as camellia oil, have shown that moderate roasting prior to pressing can enhance oxidative stability and promote the release of bioactive compounds without compromising oil quality (Hsieh *et al*. 2013).

Recent investigations have further demonstrated that roasting Sacha Inchi seeds under controlled conditions can significantly increase total phenolic content and antioxidant capacity (Kittibunchakul *et al*. 2023). Moreover, optimized roasting at 134–140℃ for 15–20 min has been shown to preserve fatty acid composition while improving oxidative stability and bioactivity (Bocanegra Morales & Galeano Garcia 2023).

To reassess the conventional cold-pressing paradigm, this study investigated the effects of thermal pre-treatment (roasting) on the chemical quality, oxidative stability, and functional properties of Sacha Inchi oil. Ten seed samples collected across Taiwan were roasted prior to being mechanically pressed without intentional heating. However, during pressing (~15 min per batch), mechanical friction raised the expeller chamber temperature to 70–80°C. The resulting oils were analyzed for acid value (AV), peroxide value (POV), oil stability index (OSI), vitamin E content, fatty acid composition, phospholipid profile, and bioactivity using cell-based anti-inflammatory assays. This study aims to determine whether controlled thermal processing can improve the functionality and stability of Sacha Inchi oil for food and nutraceutical applications.

MATERIALS AND METHODS

Plant material and sample preparation

Ten batches of mature *P. volubilis* L. (Sacha Inchi) seeds were collected from six major cultivation regions in Taiwan: Taichung, Changhua, Nantou, Chiayi, Tainan, and Hualien. Each batch was assigned a sample code (No. 1–10) for subsequent analysis. All seeds were harvested between April and May 2023, coinciding with the peak ripening season. Their fruits were harvested when the entire capsule turned brown, indicating full maturity. After harvest, local farmers sun-dried the fruits under ambient conditions to reduce their moisture content. Outer fruit hulls were manually removed, and defective fruits- such as moldy or shriveled ones- were discarded. Upon arrival at the laboratory, seed moisture content ranged from 8% to 14%. To ensure consistency across samples, all seeds were further equilibrated by hot-air oven drying at 50℃ until their moisture content fell below 10%.

The samples were then divided into two groups: one subjected to roasting, and the other retained as the unroasted control. Roasting was conducted in a convection oven at 140℃ for 20 min. All samples were mechanically dehulled to obtain kernels for oil extraction.

Oil extraction

Oil was extracted using a screw-type press expeller (Model SX-TB02, Orin Oil Press, Fad International Development, New Taipei, Taiwan) operated without intentional heating of seeds. Each batch (~15 min) was pressed at ambient conditions, during which mechanical friction raised the temperature of the expeller chamber to 70–80℃. The resulting crude oil was centrifuged at 13,690× *g* for 5 min to remove suspended solids, and the clear upper layer was collected for further analysis. To evaluate extraction efficiency, two indices were calculated:

Pressed oil ratio (%): the weight of crude oil obtained immediately after pressing divided by the weight of input seed material.

Clarified oil yield (%): the weight of centrifuged clear oil divided by the seed input weight.

Both values were expressed as weight percentages (w/w).

Oil quality

The AV and POV of each oil sample were determined according to standard American Oil Chemists' Society (AOCS) methods: Cd 3d-63 for AV and Cd 8b-90 for POV.

Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared via base-catalyzed transesterification. Approximately 0.015 g of oil was dissolved in 1 mL of *n*-hexane containing 3.0 mg of methyl heptadecanoate (internal standard; Sigma-Aldrich, St. Louis, MO, USA), followed by the addition of 60 μL of 2 N KOH in methanol. The mixture was vortexed vigorously for 5 min. To terminate the reaction, 0.4 mL of saturated sodium chloride solution was added, and the sample was centrifuged at 12,000× *g* for 2 min. The upper *n*-hexane layer was filtered through a 0.45 μm polyvinylidene fluoride (PVDF) filter (Millex-HV, Millipore, Darmstadt, Germany), diluted 100-fold, and subjected to GC-MS analysis (Varian CP-3800 GC coupled with MS 4000, Palo Alto, CA, USA).

Chromatographic separation was performed using a CP8822 column (Vf-23ms, 30 m × 0.25 mm i.d., 0.25 μm film thickness, Varian, Palo Alto, CA, USA), with an injector temperature of 240℃. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1} and a split ratio of 100 : 1. The oven temperature was initially held at 100℃ for 1 min, ramped to 185℃ at 15℃ min^{-1}, and maintained at 185℃ for 8 min. FAMEs were identified and quantified using Supelco standard mixtures (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany).

Vitamin E content

Vitamin E homologs were analyzed by high-performance liquid chromatography (HPLC) using an Inertsil ODS-3 column (4.6 mm × 250 mm, 4 μm; GL Sciences, Tokyo, Japan). The system consisted of a Waters 2695 gradient pump and a Waters 474 fluorescence detector (Milford, MA, USA). Oil samples were filtered through a 0.45 μm PVDF filter prior to injection.

A 20 μL injection volume was used, with the mobile phase composed of methanol and acetonitrile (40 : 60, v/v) in isocratic mode at a flow rate of 1 mL min^{-1}. The column temperature was maintained at 30℃. Fluorescence detection was performed at 298 nm (excitation) and 328 nm (emission). Calibration standards for α-, β-, γ-, and δ-tocopherol and tocotrienol were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

Phospholipid preparation and two-dimensional thin-layer chromatography (2D TLC) analysis

Five mL of crude oil extract were mixed with 100 μL of deionized water (2%, v/v), vortexed briefly, and shaken on a rotary shaker

(99 rpm, Intelli-Mixer RM-2L, ELMI, Riga, Latvia) at room temperature for 1 h. The mixture was centrifuged at 5,250× *g* for 15 min, and the upper oil layer was removed. The remaining white precipitate was collected as the degummed phospholipid fraction.

To further eliminate residual oil and moisture, the precipitate was centrifuged again at 5,250× *g* for 1 min, and the supernatant was discarded. This step was repeated once. The resulting pellet was dissolved in diethyl ether and dried using a freeze-dry concentrator. The dried sample was then re-dissolved in chloroform:methanol (2 : 1, v/v) for quantification and TLC analysis.

Phospholipid analysis was performed using 2D TLC, and phospholipid visualization was conducted using a modified molybdenum blue staining method, both following the protocol described in Hsieh (2017). TLC was performed using Silica Gel 60 F_{254} plates on aluminum sheets (Merck KGaA, Darmstadt, Germany).

In the first dimension, plates were developed using Solvent System I : chloroform : methanol : water (6.5 : 2.5 : 0.4, v/v/v). After drying, the plates were rotated 90 degrees and developed in the second dimension using Solvent System II: chloroform:methanol : acetic acid : water (8 : 1.2 : 1.5 : 0.4, v/v/v/v). Phospholipid standards were prepared by dissolving 10 mg of asolectin (from soybean, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) in 1 mL of chloroform : methanol (2 : 1, v/v). Dried sample pellets were re-dissolved in 25 µL of chloroform : methanol (2 : 1, v/v) and spotted onto TLC plates. Soybean asolectin was run separately as a reference standard for qualitative comparison of phospholipids.

Total phenolic content

The total phenolic content of oil samples was determined using a modified Folin-Ciocalteu method based on Singleton & Rossi (1965), as adapted by Hsieh *et al.* (2013). Briefly, oil was first diluted with isopropanol (1 : 1, v/v). A 15 µL aliquot of the mixture was added to 240 µL distilled water, 15 µL Folin-Ciocalteu reagent (Sigma-Aldrich, Buchs, Switzerland), and 30 µL of 35% aqueous sodium carbonate. The solution was vortexed thoroughly and centrifuged at 16,200× *g* for 2 min to remove undissolved oil. A 200 µL portion of the supernatant was transferred to a 96-well microplate, and absorbance was measured at 750 nm. All samples were analyzed in triplicate. Gallic acid (0–0.5 mg mL^{-1}) was used to construct the calibration curve.

Reducing power

The reducing power of oil samples was evaluated using a modified Oyaizu (1986) method, as described by Hsieh *et al.* (2013). Briefly, 100 µL of oil sample was mixed with 100 µL of 0.2 M sodium phosphate buffer (pH 6.6) and 100 µL of 1% potassium ferricyanide (prepared in buffer), then incubated at 50℃ for 20 min. The reaction was cooled on ice and terminated by adding 100 µL of 10% trichloroacetic acid. After centrifugation at 16,200× *g* for 2 min, the supernatant was collected and mixed with 100 µL distilled water and 20 µL of 0.1% ferric chloride solution. Absorbance was measured at 700 nm using a 96-well microplate reader. All samples were analyzed in triplicate. Trolox (0.08–0.8 mM), dissolved in methanol, served as the standard.

Cell culture and anti-inflammatory assay

The murine macrophage cell line RAW264.7 (BCRC, Hsinchu, Taiwan) was cultured in Dulbecco's modified eagle medium (DMEM; HyClone, Cytiva, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Cytiva, Logan, UT, USA) at 37℃ in a humidified incubator containing 5% CO_2. Sacha Inchi oil (0.4 mL) was mixed with 1 mL of absolute ethanol and centrifuged at 16,200× *g* for 20 min to obtain the ethanol-soluble fraction, herein referred to as the "29% extract." This designation is based on the volumetric proportion of oil in the total mixture (0.4 mL oil/1.4 mL total volume ≈ 29%) and reflects the treatment concentration applied in cell assays, rather than a chemical extraction yield. This extract was further diluted

with ethanol to a final concentration of 1,000 μL L^{-1} for cell treatment (the concentration was selected based on preliminary dose-response screening, data not shown), following the method of Lee *et al*. (2023). RAW264.7 cells were seeded into 48-well plates at a density of 8×10^4 cells per well and incubated for 24 h, then co-treated with lipopolysaccharide (LPS, 1 μg mL^{-1}; Sigma-Aldrich, St. Louis, MO, USA) and 1,000 μL L^{-1} of Sacha Inchi oil extract for an additional 24 h. Hydrocortisone (HC; 50 μM; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) served as a positive control. Cell-free supernatants were collected, and nitric oxide (NO) production was quantified using a modified Griess reagent (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). All treatments were performed in triplicate. The procedure was adapted from our previous protocol for fermented sweetpotato leaves (Lee *et al*. 2025), with modifications to focus exclusively on NO analysis. Statistical analyses were performed using the *t*-test function in Microsoft Excel. Data are expressed as mean ± *SD*. Significance was indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Oil stability

The oxidative stability of Sacha Inchi oil was evaluated using the OSI, which reflects the induction period (in min) before rapid oxidation occurs under accelerated thermal conditions. The analysis was conducted at a constant temperature of 130℃ using a Rancimat apparatus (Model 873 Biodiesel Rancimat, Metrohm AG, Herisau, Switzerland). For each measurement, exactly 3.00 g of oil was accurately weighed and subjected to continuous airflow at 10 L h^{-1}. The volatile oxidation products generated during heating were captured in 60 mL of distilled water, and the increase in electrical conductivity of this water was continuously recorded. The inflection point corresponding to the sharp rise in conductivity was defined as the OSI. This method allows for comparative evaluation of oxidative resistance in oil samples under uniform stress. The experimental design was based on the protocol of Hsieh *et al*. (2013), with modifications to a single testing temperature of 130℃ to simulate mild cooking conditions and to support shelf-life prediction for Sacha Inchi oil.

RESULTS AND DISCUSSION

Oil yield and sensory characteristics

Thermal pre-treatment (roasting) consistently enhanced oil extraction efficiency across ten *P. volubilis* L. (Sacha Inchi) seed samples, as evidenced by increased pressed oil ratios (Fig. 1). For all samples (No. 1–10), roasted seeds (Y) yielded a higher pressed oil ratio (%) compared to their unroasted counterparts (N), indicating that roasting facilitated oil release during cold pressing. The increase in pressed oil ratio ranged from 3% to over 25%, with the most pronounced effect observed in sample No. 5 (from 48.2% to 72.5%). Overall, 9 out of 10 samples showed improvement, with an average increase from 45.0% (unroasted) to 54.6% (roasted), representing a 21.3% relative gain.

In contrast, the clarified oil yield showed variable responses. Only 4 out of 10 samples (No. 1, 8, 9, and 10) demonstrated a net increase in clarified yield after roasting, while 6 samples (especially No. 4, 5, 6, and 7) exhibited a marked reduction. For instance, sample No. 5 had one of the largest increases in pressed oil ratio (from 50% to 73%) but suffered a significant drop in clarified yield (from 45% to 24%). These contrasting trends between pressed and clarified yields underscore the dual impact of roasting- enhancing oil release while potentially complicating post-extraction clarification.

To summarize the impact of roasting on oil yield, Table 1 presents the average values of the pressed oil ratio and clarified oil yield across all 10 samples. While roasting consistently improved the pressed oil ratio, the clarified oil yield exhibited a less uniform response. This divergence suggests that although roasting promotes oil liberation, it may also increase the presence of suspended solids, emulsifying

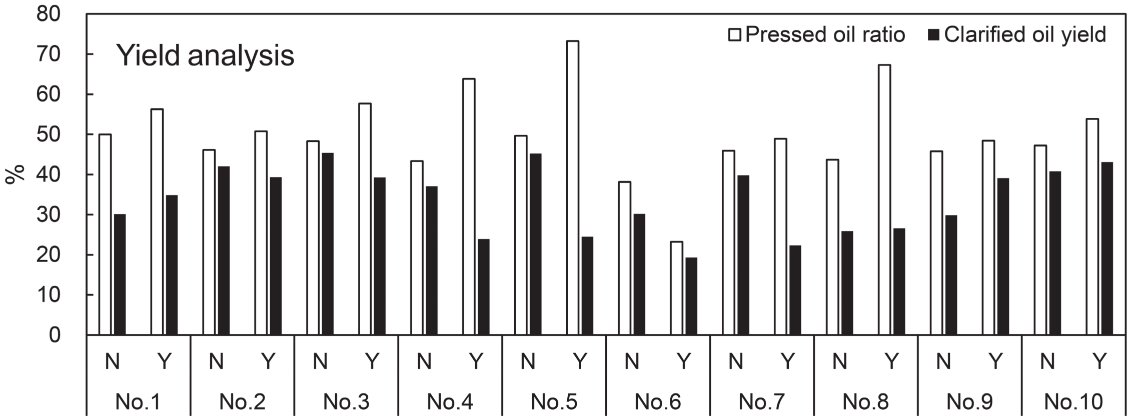


Fig. 1. Yield analysis of Sacha Inchi oil extracted from ten seed samples subjected to roasting pre-treatment (Y) or retained as unroasted controls (N). Pressed oil ratio (%) indicates the proportion of crude oil obtained by mechanical pressing relative to seed input. Clarified oil yield (%) refers to the percentage of centrifuged clear oil recovered from the pressed fraction. Roasting generally increased the pressed oil ratio across most samples, whereas its effect on clarified oil yield was more variable. Summary statistics are provided in Table 1.

Table 1. Average oil yield performance with and without roasting treatment[z].

Yield parameter	Unroasted (N, mean)	Roasted (Y, mean)	Mean difference	Observation
Pressed oil ratio (%)	45.0	54.6	9.6	Increased in 9 out of 10 samples
Clarified oil yield (%)	35.6	33.3	-2.3	Increased in 4 samples, decreased in 6

[z] Values represent mean percentages across 10 independent samples per treatment.

agents (such as phospholipids and denatured proteins), or thermally induced particulates that hinder efficient oil clarification by centrifugation. Similar observations have been reported in peanut systems, where roasting-induced emulsifying agents and heat-generated particulates interfered with oil separation efficiency (Suri *et al.* 2019; Zhang *et al.* 2023).

Interestingly, some samples, such as No. 9 and No. 10 showed improvements in both pressed and clarified yields post-roasting, suggesting that seed characteristics (e.g., initial moisture content or protein composition) may modulate the balance between oil release and sediment formation. These findings emphasize the need to optimize roasting parameters based on seed traits to balance extraction efficiency and oil clarity. Further compositional analysis of the sediment may clarify the mechanism.

In addition to yield performance, sensory evaluation revealed distinct differences in flavor profiles between unroasted and roasted oils. Unroasted Sacha Inchi oil was often described as having raw or grassy notes, which may be less appealing to consumers. In contrast, roasted oils exhibited pleasant peanut-like and nutty aromas, thereby improving flavor acceptability. These sensory enhancements further support the application of roasting as a value-adding step in cold-pressed oil production.

Oil quality: AV and POV

Oil quality was assessed using AV and POV, which represent hydrolytic and oxidative stability, respectively. AV ranged from 0.10 to 0.50 mg KOH g^{-1} in unroasted samples and from 0.10 to 0.70 mg KOH g^{-1} in roasted oils (Fig. 2A; Table 2). AV increased slightly in 7 out of 10 samples, with a maximum change of + 0.17 mg KOH g^{-1} (e.g., No. 1). Despite this increase, all values remained well below the Codex Alimentarius threshold of 2.0 mg

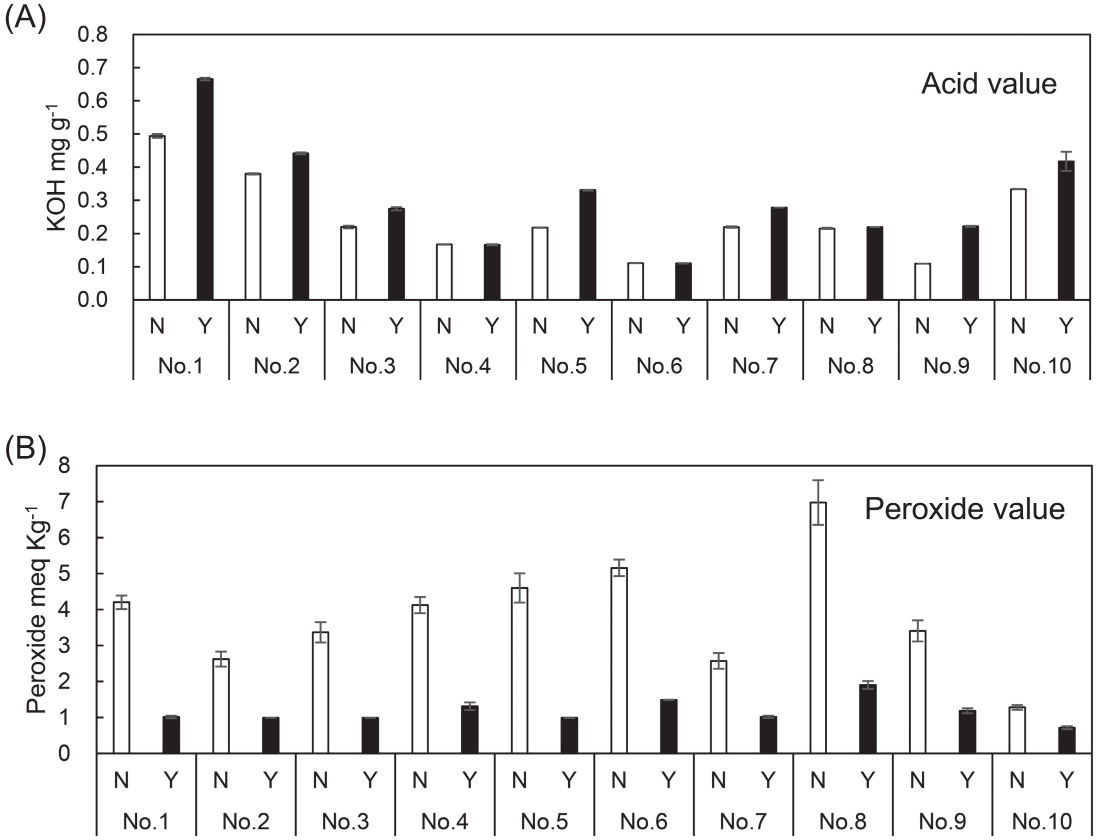


Fig. 2. Oil quality parameters of Sacha Inchi oil. (A) Acid value (AV) and (B) peroxide value (POV) of oils extracted from ten seed samples processed under unroasted (N) and roasted (Y) conditions. AV values showed slight increases after roasting but remained well within acceptable limits for edible oils. In contrast, POV values decreased markedly in all roasted samples, indicating enhanced oxidative stability associated with roasting (summary statistics provided in Table 2). Values represent mean ± *SD* of duplicate and tetraplicate measurements for AV and POV, respectively.

Table 2. AV[z] and POV[y] summary.

Quality parameter	Unroasted (N)	Roasted (Y)	Change trend
AV (mean[x], mg KOH g^{-1})	0.29	0.37	Increased in 7 samples, decreased in 3
POV (mean, meq O_2 kg^{-1})	3.65	1.16	Decreased in all samples

[z] Acid value.
[y] Peroxide value.
[x] Values represent mean measurements across 10 independent samples per treatment.

KOH g^{-1} for Sacha Inchi oil, as endorsed by the Codex Committee on Fats and Oils and submitted for final adoption at CAC47, thereby confirming that moderate roasting did not induce significant hydrolysis.

In contrast, POV showed a consistent reduction across all roasted samples (Fig. 2B). Unroasted oils exhibited POVs ranging from 1.28 to 6.98 meq O_2 kg^{-1}, while roasted counterparts ranged from 0.71 to 1.90 meq O_2 kg^{-1}. The average POV decreased from 3.65 to 1.16 meq O_2 kg^{-1}, representing a 68% reduction. This is well below the Codex recommended limit of 15 meq O_2 kg^{-1} for virgin Sacha Inchi oil, suggesting

that roasting not only avoided oxidation but actively reduced pre-existing peroxides, thereby improving oil stability.

These results indicate that controlled moderate roasting does not compromise oil quality and may enhance oxidative resilience. This finding challenges the common perception that PUFA-rich oils must be strictly cold-pressed to avoid degradation. Similar improvements have been reported in other oilseeds, such as camellia oil, where moderate roasting improved oxidative parameters without compromising lipid integrity (Hsieh *et al*. 2013).

Unaltered nutrients under roasting: Vitamin E and fatty acids

The nutritional stability of Sacha Inchi oil during roasting was evaluated by analyzing vitamin E homologs and fatty acid composition. The vitamin E profile was dominated by γ-tocopherol, followed by δ-tocopherol and α-tocotrienol (Fig. 3A). Mean concentrations of γ-T, δ-T, and α-T_3 were comparable between unroasted (779, 775, and 85 mg kg^{-1}) and roasted oils (784, 773, and 84 mg kg^{-1}), respectively. Minor fluctuations between samples- such as a slight decrease in δ-T in sample No. 3 and a slight increase in α-T_3 in No.10- fell within

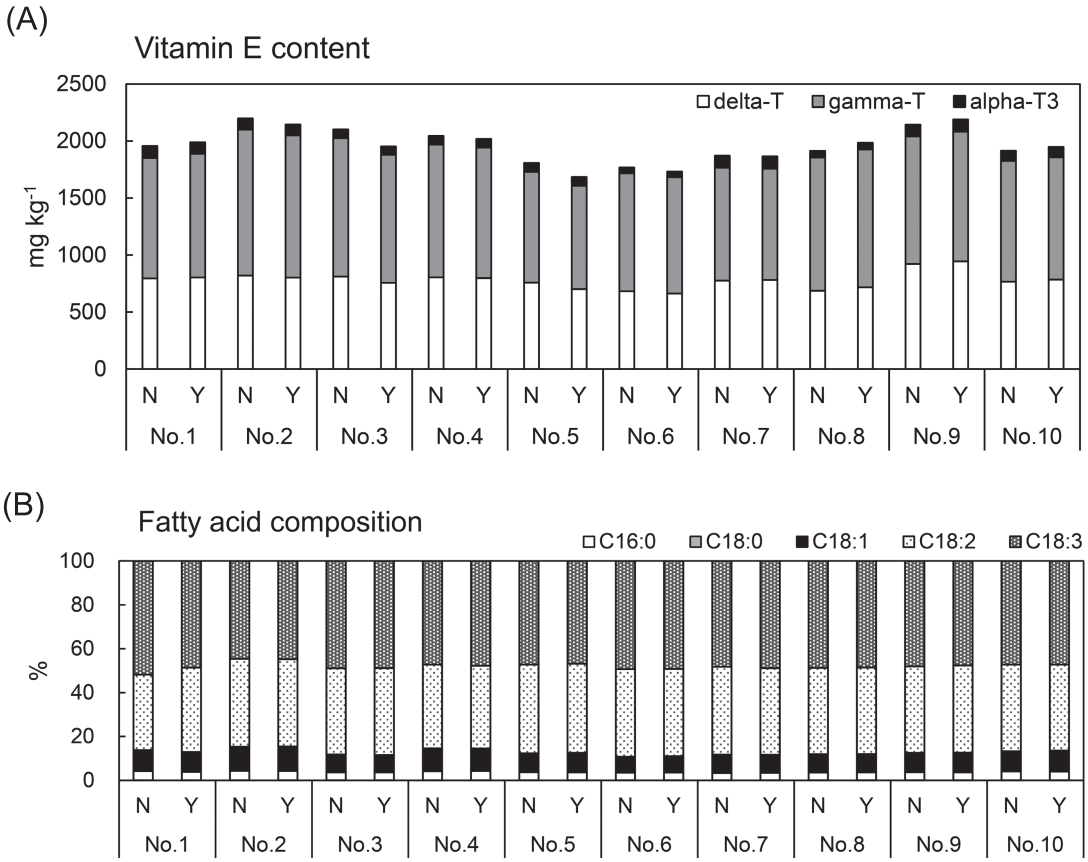


Fig. 3. Composition of functional components in Sacha Inchi oil under unroasted (N) and roasted (Y) conditions. (A) Vitamin E homologs, including δ-tocopherol (δ-T), γ-tocopherol (γ-T), and α-tocotrienol (α-T_3). (B) Fatty acid profile, including palmitic acid (C16 : 0), stearic acid (C18 : 0), oleic acid (C18 : 1), linoleic acid (C18 : 2), and α-linolenic acid (C18 : 3). No significant differences were observed between treatments, indicating compositional stability of both lipid and antioxidant components following roasting. Values are represented with duplicate measurements.

normal biological variability, and no systematic trend was observed.

Similarly, the fatty acid composition remained largely unaffected by roasting (Fig. 4B). The major components were α-linolenic acid (C18 : 3, 46–52%), linoleic acid (C18 : 2, 34–40%), and oleic acid (C18 : 1, 7–11%). Saturated fatty acids, mainly palmitic acid (C16 : 0), ranged from 3.4% to 4.3%, with negligible levels of stearic acid (C18 : 0 < 0.1%). The consistently high PUFA content (> 85%) in both treatments reinforces the nutritional value of Sacha Inchi oil as a rich source of ω-3 fatty acids, even after thermal exposure.

These results indicate that moderate roasting does not degrade vitamin E homologs or alter the oil's core lipid profile. This stability contrasts with the commonly assumed heat sensitivity of polyunsaturated oils. The findings support the feasibility of incorporating controlled roasting in Sacha Inchi oil production, balancing processing efficiency with nutritional preservation, as similarly observed in heat-treated oilseeds (Hsieh *et al.* 2013).

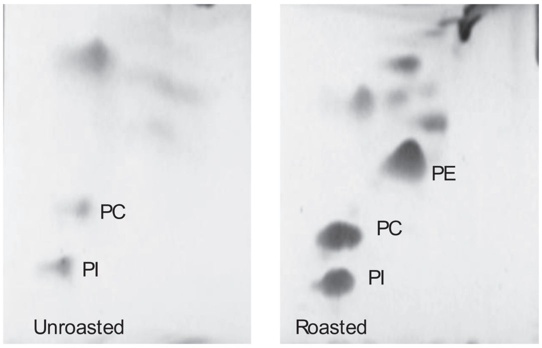


Fig. 4. Thin-layer chromatographic (TLC) analysis of phospholipid composition in Sacha Inchi oil under unroasted and roasted conditions. Representative TLC plates from a Taichung-sourced sample illustrate the separation of three major phospholipids: phosphatidylinositol (PI), phosphatidylcholine (PC), and phosphatidylethanolamine (PE). Roasting visibly enhanced the diversity and intensity of phospholipid bands, particularly the emergence of PE. Representative TLC image shown (Taichung sample); all other batches exhibited consistent spot patterns with minor differences in intensity or background.

Roasting-induced functional enhancement

1. Enriched bioactive compounds: Phospholipids and phenolics

Roasting-induced changes in the bioactive composition of Sacha Inchi oil were examined, with a focus on phospholipids and phenolic compounds. Phospholipid profiles were assessed using TLC, based on a representative sample sourced from Taichung, Taiwan. This sample was selected for its consistent compositional traits across batches and serves as a visual example to illustrate the impact of roasting on lipid diversity (Fig. 4). Three major phospholipids were identified: phosphatidylinositol (PI), phosphatidylcholine (PC), and phos-phatidylethanolamine (PE).

In the unroasted oil, only PI and PC bands were clearly visible, while PE was faint or undetectable. In contrast, roasted oil exhibited all three phospholipids with distinct separation and visibly stronger band intensity, particularly for PE. This qualitative shift suggests that roasting promotes the release of membrane-associated phospholipids, resulting in a more diverse lipid profile.

The emergence of PE in roasted oil is especially noteworthy given its potential antioxidant activity. Although native PE exhibits limited radical-scavenging activity, recent studies have shown that thermally generated PE-derived Maillard reaction products (MRPs) can significantly enhance lipid oxidative stability (Shrestha & De Meulenaer 2014; Li *et al.* 2024). These findings suggest that roasting may not only release PE but also promote the formation of functional derivatives that contribute to the improved oxidative stability of roasted Sacha Inchi oil. While TLC does not provide precise quantification, the visual enhancement of phospholipid bands supports the conclusion that roasting modifies lipid composition in a functionally meaningful way.

In addition to changes in phospholipids, roasting significantly increased the total phenolic content of Sacha Inchi oil. Across ten seed samples, unroasted oils exhibited phenolic concentrations

ranging from 0.023 to 0.046 mg gallic acid equivalents (GAE) mL^{-1}, whereas roasted oils ranged from 0.055 to 0.124 mg GAE mL^{-1} (Fig. 5A). The most pronounced increase was observed in sample No. 8, where roasting elevated phenolic content by 170%. On average, phenolic levels increased from 0.035 ± 0.007 mg GAE mL^{-1} (unroasted) to 0.082 ± 0.018 mg GAE mL^{-1} (roasted), representing a 2.3-fold enhancement.

This consistent upward trend suggests that moderate roasting facilitates the release or transformation of bound phenolic compounds,

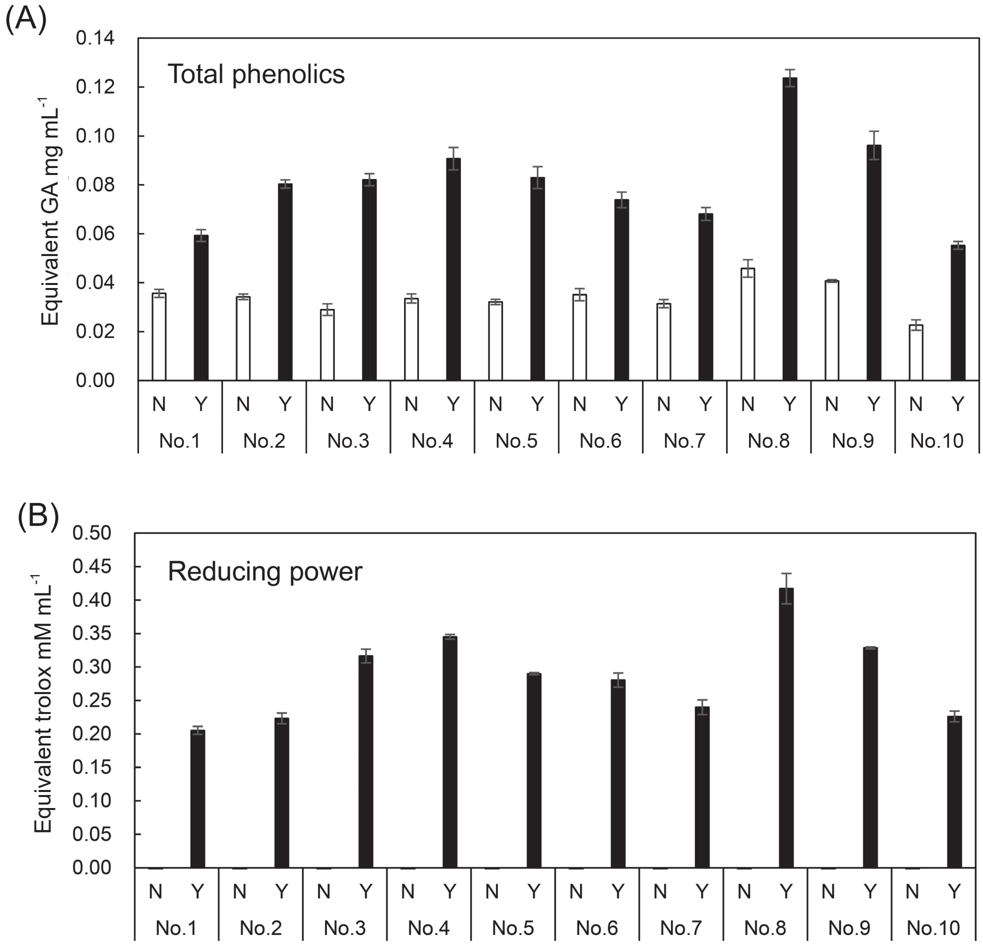
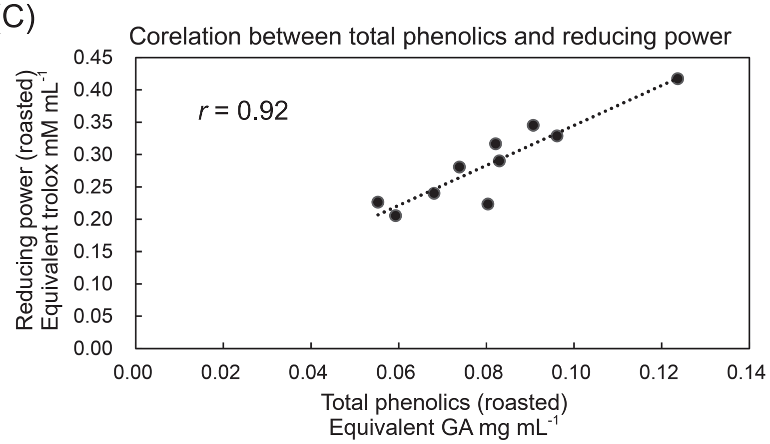
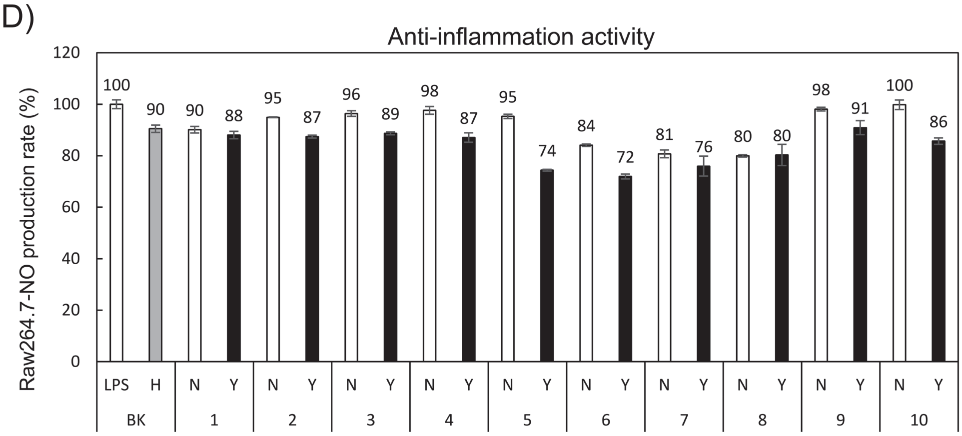


Fig. 5. Functional compound enrichment and bioactivity enhancement in roasted Sacha Inchi oil. (A) Total phenolic content of Sacha Inchi oil under unroasted and roasted conditions. Roasting consistently increased phenolic levels across all ten seed samples, with values rising from near-baseline to over 0.12 mg gallic acid equivalents (GAE) mL^{-1} in representative cases. (B) The reducing power of Sacha Inchi oil under unroasted and roasted conditions. Roasted samples exhibited measurable antioxidant activity, while unroasted oils remained near or below baseline, indicating negligible electron-donating capacity prior to thermal treatment. (C) Correlation between total phenolic content and reducing power in roasted samples. A strong positive relationship (r = 0.92) was observed, suggesting phenolic enrichment contributes directly to antioxidant functionality. (D) Inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages following oil treatment. Both roasted and unroasted oils reduced NO levels relative to the LPS-only control (set at 100%), with several roasted samples (e.g., No. 5–7) exhibiting stronger inhibition than the positive control hydrocortisone (H). Statistical comparisons between unroasted and roasted treatments for each sample are summarized in Table 3. P-values were calculated by paired t-test comparing roasted (Y) and unroasted (N) treatments for each sample (n = 3). Values represent mean ± SD of triplicate measurements. BK: blank control.

(C)



(D)



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possibly through cell wall disruption or the formation of Maillard reaction intermediates. Recent studies have shown that Maillard-derived α-dicarbonyl compounds and Amadori rearrangement products can enhance phenolic solubility and reactivity by forming conjugates or adducts with flavonoids and hydroxycinnamic acids, thereby increasing their extractability and antioxidant potential (Han *et al.* 2024).

2. Enhanced functional activities: Antioxidant capacity and NO inhibition

To further evaluate the functional impact of roasting on Sacha Inchi oil, antioxidant and anti-

inflammatory activities were assessed through reducing power assays and NO inhibition tests. The results are summarized in Figs. 5A–D, which illustrate the enrichment of phenolic compounds, the emergence of reducing power, the correlation between these two parameters, and the suppression of NO production in macrophage cells.

Antioxidant capacity was evaluated using reducing power assays across 10 seed samples, expressed as trolox-equivalent concentrations (Equi. trolox mM mL^{-1}). As shown in Fig. 5B, unroasted oils exhibited values near or below baseline (-0.065 mM mL^{-1} to -0.009 mM mL^{-1}), indicating negligible electron-donating activity and the absence of measurable reducing capability. In contrast, roasted oils demonstrated distinctly positive values (0.205 mM mL^{-1} to 0.417 mM mL^{-1}), reflecting the activation of antioxidant functionality. Sample No. 8 showed the strongest response, transitioning from baseline to 0.417 mM. Overall, roasted samples consistently exhibited measurable reducing power, confirming that thermal treatment initiates antioxidant activity otherwise absent in native oil.

A correlation analysis between total phenolic content and reducing power in roasted samples revealed a strong positive relationship (r = 0.92), as shown in Fig. 5C. This suggests that phenolic enrichment is a key contributor to the antioxidant functionality observed after roasting. These findings support the hypothesis that moderate roasting enhances antioxidant capacity primarily through phenolic activation. Maillard reaction intermediates have also been reported to improve phenolic solubility and reactivity, further contributing to antioxidant enhancement (Han *et al*. 2024).

The anti-inflammatory potential of Sacha Inchi oil was evaluated by measuring NO production in LPS-stimulated RAW264.7 macrophages following oil treatment. As shown in Fig. 5D, all oil samples- both roasted and unroasted- exhibited inhibitory effects compared to the LPS-only control (set at 100%). Hydrocortisone (H), used as a positive control, reduced NO levels to 90.5%. Most oil-treated groups showed comparable or stronger inhibition than HC. Notably, roasted samples No. 5, 6, and 7 reduced NO production to 74.4%, 71.9%, and 75.9%, respectively- substantially lower than both HC and their unroasted counterparts.

Statistical significance was assessed using two approaches: one-sample t-tests comparing each treatment group to the LPS control, and paired t-tests comparing roasted (Y) versus unroasted (N) extracts within each sample. Significant reductions in NO production ($P < 0.05$) were observed in most roasted samples, particularly No. 2, 3, 5, 6, 7, 8, and 9 (Table 3). In addition, paired comparisons revealed that roasting significantly enhanced NO inhibition in 9 out of 10 samples, with P-values ranging from 0.0490 to < 0.0001. These results confirm that roasting consistently improves the anti-inflammatory activity of Sacha Inchi oil.

The enhanced inhibition may reflect thermally induced release or transformation of bioactive compounds such as phenolics and phospholipids (e.g., PE), which have been associated with immunomodulatory effects (van Dieren *et al*. 2011). Although sample No. 8 exhibited the highest phenolic content and strongest reducing power, its NO inhibition was only moderate, suggesting that the anti-inflammatory effects of Sacha Inchi oil may involve additional pathways beyond antioxidant mechanisms. Overall, samples with stronger NO inhibition tended to show elevated phenolic content and reducing power, supporting the functional relevance of roasting in enhancing both antioxidant and anti-inflammatory properties of Sacha Inchi oil.

3. Improved oxidative stability: Rancimat resistance and nutrient synergy

To simulate mild cooking scenarios and evaluate the thermal oxidative stability of Sacha Inchi oil, the OSI was measured using the Rancimat method at 130℃ (Fig. 6). OSI represents the induction period length and reflects the oil’s resistance to oxidative degradation under accelerated heating conditions. This temperature

Table 3. Effects of roasted and unroasted Sacha Inchi oil extracts on nitric oxide (NO) production in LPS-stimulated RAW264.7 cells and statistical significance.

Sample No.	Treatment[z]	NO production (% of LPS)	*P*-value[y] vs. LPS (significance)	Mean difference N–Y	*P*-value N vs Y (significance)
BK[x]	LPS	100.0 ± 1.7	1.0000 (-)	-	-
	H	90.5 ± 1.4	0.0020 (**)	-	-
No. 1	N	90.1 ± 1.3	0.0017 (**)	0.0215	0.0490 (*)
	Y	88.0 ± 1.4	0.0008 (***)		
No. 2	N	94.9 ± 0.2	0.0344 (*)	0.0752	0.0010 (**)
	Y	87.4 ± 0.6	0.0030 (**)		
No. 3	N	96.4 ± 1.1	0.0445 (*)	0.0770	0.0000 (***)
	Y	88.7 ± 0.6	0.0039 (**)		
No. 4	N	97.6 ± 1.5	0.1469 (NS)	0.1059	0.0000 (***)
	Y	87.0 ± 1.8	0.0009 (***)		
No. 5	N	95.3 ± 0.8	0.0244 (*)	0.2085	0.0000 (***)
	Y	74.4 ± 0.3	0.0010 (***)		
No. 6	N	84.1 ± 0.4	0.0023 (**)	0.1213	0.0000 (***)
	Y	71.9 ± 0.9	0.0001 (***)		
No. 7	N	80.7 ± 1.5	0.0001 (***)	0.0478	0.0010 (**)
	Y	75.9 ± 3.9	0.0032 (**)		
No. 8	N	79.9 ± 0.4	0.0014 (**)	-0.0036	0.7720 (NS)
	Y	80.3 ± 4.1	0.0071 (**)		
No. 9	N	98.1 ± 0.7	0.1837 (NS)	0.0721	0.0000 (***)
	Y	90.9 ± 2.7	0.0124 (*)		
No. 10	N	99.8 ± 1.8	0.9068 (NS)	0.1414	0.0000 (***)
	Y	85.7 ± 1.2	0.0005 (***)		

[z] LPS: lipopolysaccharide-stimulated control group (set at 100% NO production); H: hydrocortisone-treated positive control; N: oil extract from unroasted Sacha Inchi seeds; Y: oil extract from roasted Sacha Inchi seeds.

[y] *P*-values (N vs Y) were calculated by paired *t*-test comparing roasted (Y) and unroasted (N) treatments for each sample (n = 3). Statistical analyses were performed with the *t*-test function in Microsoft Excel. Data are expressed as mean ± *SD*. Significance are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; NS: not significant.

[x] BK: blank control.

setting was chosen to mimic practical culinary applications such as light sautéing or warm salad preparation, offering real-world relevance.

The results showed that roasted samples consistently exhibited longer induction periods than their unroasted counterparts, demonstrating stable and reproducible oxidative resistance. OSI values for unroasted oils clustered between 8 min and 11 min, indicating the inherent susceptibility of polyunsaturated oils to oxidation in their native state. In contrast, roasted oils showed extended OSI values ranging from 21 to 53 min, confirming that moderate roasting significantly enhances heat stability.

Among all samples, No. 10 exhibited the most pronounced improvement, with OSI increasing from 11 to 53 min- a nearly 5-fold enhancement. Other notable increases were observed in sample No. 6 (from 10 to 36 min), No. 2 (from 10 to 35 min), and No. 8 (from 10 to 43 min). On average, unroasted oils had an OSI of approximately 10 min, while roasted oils reached an average of 33 min, indicating a consistent and substantial improvement in thermal oxidative stability following roasting.

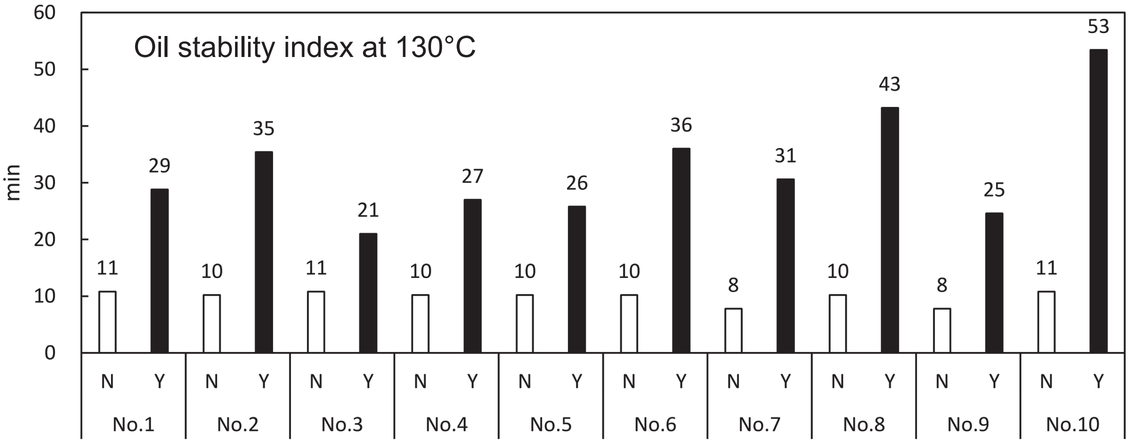

Fig. 6. Oxidative stability of Sacha Inchi oil under unroasted and roasted conditions. Oil stability index (OSI) values were measured using the Rancimat method at 130℃ to simulate light cooking conditions. Roasted samples consistently exhibited longer induction times than their unroasted counterparts, indicating improved thermal oxidative resistance. Sample No. 10 showed the most pronounced enhancement, increasing from 11 to 53 min. The average OSI increased from 10 min (unroasted) to approximately 33 min (roasted), supporting the functional benefit of controlled roasting.

This enhancement may result from multiple mechanisms, including enzyme inactivation (e.g., lipoxygenase; Zheng *et al*. 2023), moisture reduction, and the release or activation of endogenous antioxidants such as phenolics and phospholipids. Thermal stabilization has been shown to effectively suppress lipoxygenase activity and promote antioxidant release in lipid-rich matrices, thereby improving oxidative resilience. These mechanisms not only stabilize the oil matrix but also correspond with the previously observed improvements in reducing power (Hsieh *et al*. 2013, 2014). Furthermore, the earlier-documented decrease in POV reinforces this conclusion, confirming that moderate thermal treatment can effectively enhance the oxidative stability of Sacha Inchi oil.

Taken together, controlled roasting emerges as a practical strategy to enhance oil quality and extend shelf life in both food and nutraceutical applications. Beyond oxidative stability, roasting may also promote nutrient synergy by increasing the bioavailability and interaction of endogenous antioxidants such as phenolics and phospholipids, thereby strengthening the oil's functional performance. These compounds not only stabilize the lipid matrix but also help mitigate inflammatory responses, supporting the health-promoting potential of Sacha Inchi oil. The observed improvement in heat stability at 130℃ further suggests that roasted oil is suitable for mild cooking applications, such as sautéing or warm salad preparation, offering practical value while preserving bioactivity.

Integrated interpretation of roasting effects

The roasting temperature of 140℃ for 20 min was selected based on prior studies in sesame (*Sesamum indicum*), which demonstrated optimal flavor and functional compound retention under similar conditions (Huang *et al*. 2017). Preliminary trials in Sacha Inchi further confirmed that this condition preserved fatty acid integrity while enhancing phenolic content and oxidative stability. Accordingly, this thermal pre-treatment was adopted as the controlled roasting condition in this study.

Roasting under defined thermal conditions (140℃, 20 min) induced changes in the chemical composition, oxidative stability, and biofunctional properties of Sacha Inchi oil. These effects extended beyond isolated improvements, reflect-

ing a systemic enhancement of oil quality driven by physical and biochemical transformations.

From a chemical standpoint, roasting had minimal impact on AV, which remained within Codex Alimentarius specifications (AV < 2), indicating no significant hydrolytic degradation. In contrast, POV decreased substantially, suggesting that roasting either eliminated pre-existing peroxides or delayed further oxidation. The OSI at 130℃ increased by 2–5-fold in most samples, clearly demonstrating enhanced resistance to thermal oxidation.

Functionally, roasting preserved the fatty acid composition and vitamin E profile, maintaining essential lipid nutrition. Meanwhile, it significantly elevated phospholipids, total phenolic content, reducing power, and anti-inflammatory activity- indicating a functional upgrade likely attributable to heat-induced release of bound antioxidants or the formation of novel antioxidative compounds, such as Maillard-derived phenolics. Phospholipid profiling revealed the emergence of PE, a compound absent in unroasted oil, which may contribute to emulsion stability and oxidative defense.

Taken together, these findings suggest that controlled roasting induces favorable chemical and structural changes- enhancing oil extractability, increasing antioxidant potential, and improving oxidative stability- without compromising the core nutritional profile. Roasting thus offers a practical, value-adding step in Sacha Inchi oil production, particularly for food and nutraceutical applications requiring both stability and bioactivity.

Nevertheless, this study was limited by the use of a single roasting condition (140℃, 20 min) and a laboratory scale capacity (each batch used approximately 300 g of seeds, which is sufficient for chemical and functional analyses). Future studies should expand the range of roasting parameters to evaluate their effects on oil quality, biofunctional properties, and long term storage stability. Moreover, large scale production may introduce additional variability due to differences in expeller models, friction induced heating, and batch volumes; therefore, processing conditions across different expeller types should be examined to enhance generalizability and industrial relevance.

ACKNOWLEDGEMENTS

This study was supported by the Council of Agriculture, Taiwan, ROC (Grant Nos. 111AS-11.1.2-CI-C2, 112AS-4.2.7-CI-C1, and 113AS-4.2.2-CI-02). The authors thank Chiu-Hua Chen for her assistance with cell culture and NO analysis.

REFERENCES

Bocanegra Morales, N. and P. Galeano Garcia. 2023. Chemical composition, fatty acid profile, and optimization of the Sacha Inchi (*Plukenetia volubilis* L.) seed-roasting process using response surface methodology: Assessment of oxidative stability and antioxidant activity. Foods 12:3405. doi:10.3390/foods12183405

Bussmann, R. W., N. Paniagua Zambrana, and C. Téllez. 2013. *Plukenetia carolis-vegae* (Euphorbiaceae)- A new useful species from northern Peru. Econ. Bot. 67:387–392. doi:10.1007/s12231-013-9247-2

Codex Alimentarius Commission (CAC). 2021. Amendment/revision to the codex Standard for Named Vegetable Oils (CXS 210-1999)- Inclusion of Sacha Inchi oil. https://media.maiagent.ai/media/chat/attachment/24fdd43a-90a8-4910-8043-768e8e660fea.pdf (visit on 10/14/2025)

Codex Alimentarius Commission (CAC). 2024. Proposed draft amendment to the Standard for Named Vegetable Oils (CXS 210-1999): Inclusion of Sacha Inchi oil. https://reurl.cc/j6vkb2 (visit on 10/14/2025)

Han, Z., M. Zhu, X. Wan, X. Zhai, C. T. Ho, and L. Zhang. 2024. Food polyphenols and Maillard reaction: Regulation effect and chemical mechanism. Crit. Rev. Food Sci. Nutr. 64:4904–4920. doi:10.1080/10408398.2022.2146653

Hsieh, C. M., J. C. Yang, D. S. Chen, Y. C. Chuang, E. I. C. Wang, and Y. L. Lee. 2014. Effects of roasting *Camellia tenuifolia* seeds with/without shells on the quality of seed oil. J. Taiwan Agric. Res. 63:17–29. doi:10.6156/JTAR/2014.06301.02

Hsieh, C. M., J. C. Yang, Y. C. Chuang, E. I. C. Wang, and Y. L. Lee. 2013. Effects of roasting prior to pressing on the camellia oil quality. J. Taiwan Agric. Res. 62:249–258. doi:10.6156/JTAR/2013.06203.05

Hsieh, T. Y. 2017. Characterization of novel species bacteria of *Paracoccus*, *Ideonella*, *Undibacterium*, *Neptunomonas*, *Hymenobacter* and *Mucilaginibacter* isolated from Taiwan waters area. Master Thesis. Department of Seafood Science, National Kaohsiung Marine University. Kaohsiung, Taiwan. 252 pp. (in Chinese with English abstract)

Huang, R. C., H. C. Tso, C. Chu, and Y. L. Lee. 2017. Effects of roasting conditions on the functional components of sesame. J. Taiwan Agric. Res. 66:113–125. doi:10.6156/JTAR/2017.06602.04

Kittibunchakul, S., V. Kemsawasd, C. Hudthagosol, P. Hudthagosol, S. Sapwarobol, and U. Suttisansanee. 2023. The effects of different roasting methods on the phenolic contents, antioxidant potential, and *in vitro* inhibitory activities of Sacha Inchi seeds. Foods 12:4178. doi:10.3390/foods12224178

Lee, Y. L., C. L. Huang, T. H. Hung, W. T. Liu, S. Y. Lin, C. H. Chen, … C. E. Tseng. 2025. Phytochemicals and physiological activities of sweetpotato (*Ipomoea batatas* L.) leaves processed via full fermentation. J. Taiwan Agric. Res. 74:11–23. doi:10.6156/JTAR.202503_74(1).0002

Lee, Y. L., W. T. Liu, T. H. Hung, S. Y. Lin, Y. H. Ho, Y. F. Lee, … P. J. Chang. 2023. Influence of drying methods of Camellia seeds on the quality and bioactive ingredients of the pressed oils. J. Taiwan Agric. Res. 72:39–48. (in Chinese with English abstract) doi:10.6156/JTAR.202303_72(1).0004

Li, K., Y. Du, Q. Xiong, N. Zhang, Z. Cai, and Y. Wang. 2024. Preparation and antioxidant activity of Maillard reaction products from phosphatidylethanolamine and reducing sugars. China Oils Fats 49(4):65–71, 76. (in Chinese with English abstract) doi:10.19902/j.cnki.zgyz.1003-7969.220838

Mhd Rodzi, N. A. R. and L. K. Lee. 2022. Sacha Inchi (*Plukenetia volubilis* L.): Recent insight on phytochemistry, pharmacology, organoleptic, safety and toxicity perspectives. Heliyon 8:e10572. doi:10.1016/j.heliyon.2022.e10572

Mhd Rodzi, N. A. R., M. Mohd Sopian, and L. K. Lee. 2025. Effects of Sacha Inchi (*Plukenetia volubilis* L.) oil supplementation on hyperglycaemia, hypertension and hyperlipidaemia (3Hs) patients: A preliminary human trial. Plant Foods Hum. Nutr. 80:80. doi:10.1007/s11130-025-01309-8

Oyaizu, M. 1986. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307–315. (in Japanese with English abstract) doi:10.5264/eiyogakuzashi.44.307

Shrestha, K. and B. De Meulenaer. 2014. Antioxidant activity of Maillard type reaction products between phosphatidylethanolamine and glucose. Food Chem. 161:8–15. doi:10.1016/j.foodchem.2014.03.113

Singleton, V. L. and J. A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Amer. J. Enol. Vitic. 16:144–158. doi:10.5344/ajev.1965.16.3.144

Suri, K., B. Singh, M. Kaur, and N. Singh. 2019. Impact of roasting and extraction methods on chemical properties, oxidative stability and Maillard reaction products of peanut oils. J. Food Sci. Technol. 56:2436−2445. doi:10.1007/s13197-019-03719-4

van Dieren, J. M., Y. Simons-Oosterhuis, H. C. Raatgeep, D. J. Lindenbergh-Kortleve, M. E. H. Lambers, C. J. van der Woude, … E. E. S. Nieuwenhuis. 2011. Anti-inflammatory actions of phosphatidylinositol. Eur. J. Immunol. 41:1047–1057. doi:10.1002/eji.201040899

Zhang, Y., Y. Chen, C. Liu, F. Cheng, and L. Yin. 2023. Effects of roasting temperatures on peanut oil and protein yield extracted via aqueous enzymatic extraction and stability of the oil body emulsion. Foods 12:4183. doi:10.3390/foods12224183

Zheng, Q., Z. Wang, F. Xiong, and G. Zhang. 2023. Enzyme inactivation induced by thermal stabilization in highland barley and impact on lipid oxidation and aroma profiles. Front. Nutr. 10:1097775. doi:10.3389/fnut.2023.1097775

適度烘焙處理提升印加果 (*Plukenetia volubilis* L.) 油生理活性與穩定性並不改變其脂肪酸組成

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摘要

李雅琳、洪子桓、劉威廷、林素月、何奕漢、李穎甫。2026。適度烘焙處理提升印加果 (*Plukenetia volubilis* L.) 油生理活性與穩定性並不改變其脂肪酸組成。台灣農業研究 75(2):333–349。

印加果 (*Plukenetia volubilis* L.) 油富含 ω-3 脂肪酸與維生素 E，具備潛在健康效益，近年來備受全球關注。然而，其氧化穩定性低，成為商業應用上的主要限制。本研究採用中度烘焙熱前處理，比較烘焙與未烘焙種子的榨出油脂，對於印加果油化學組成、氧化穩定性及功能活性的影響。本研究共收集臺灣 6 個地區 (臺中、彰化、南投、嘉義、臺南、花蓮) 之 10 件種子樣品，經去殼後以不加熱的物理壓榨法萃取油品。分析項目包括酸價 (acid value; AV)、過氧化價 (peroxide value; POV)、油脂氧化安定性指數 (oil stability index; OSI)，於 130℃測定)、維生素 E 含量、脂肪酸組成、磷脂組成 (以薄層層析法分析)、總酚含量、還原力，以及以細胞模式評估之抗發炎活性。結果顯示，烘焙處理未改變脂肪酸與維生素 E 組成，但顯著提升氧化安定性 (OSI 上升)，並促進磷脂 (如磷胺酯) 與總酚等生物活性成分增加並伴隨還原力增強。烘焙與未烘焙油品皆具備抗發炎活性，其中烘焙油在抑制脂多醣 (lipopolysaccharide; LPS) 刺激巨噬細胞產生一氧化氮 (nitric oxide; NO) 的反應表現略優。綜合而言，適度烘焙處理可以有效提升印加果油之氧化安定性、生物活性組成及生理功能性，同時保留其核心營養價值 (ω-3 脂肪酸與維生素 E)。本研究結果支持烘焙作為提升印加果油品質之可行加工策略，拓展其在食品與營養保健領域之應用潛力。

關鍵詞：印加果油、烘焙、氧化安定性、功能性生物活性、抗發炎活性。

投稿日期：2025 年 10 月 14 日；接受日期 2025 年 12 月 5 日。
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台灣農業研究 (J. Taiwan Agric. Res.) 75(2):351–363 (2026)
https://doi.org/10.6156/JTAR.202606_75(2).0013

Research Article

Development of a Genetic Structure-Based Core Collection in *Capsicum* spp. for Efficient Germplasm Utilization

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Abstract

Li, C. B., J. Y. Wang, D. G. Lin, C. C. Chen, C. Y. Lin, Y. K. Tu, C. P. Kuan, and S. Y. Lin. 2026. Development of a genetic structure-based core collection in *Capsicum* spp. for efficient germplasm utilization. J. Taiwan Agric. Res. 75(2):351–363.

We developed a flexible core collection of pepper (*Capsicum* spp.) germplasm from the National Genebank of Taiwan and demonstrated its utility in screening for resistance to pepper mild mottle virus (PMMoV). A total of 1,011 accessions were sequenced, revealing four distinct genetic clusters associated with specific phenotypic traits. Population structure analysis confirmed the strong correspondence between genotypic and phenotypic variation, reflecting the association between population genetic structure and trait expression. Linkage disequilibrium (LD) decay analysis across major *Capsicum* species provided insights into population differentiation and informed single-nucleotide polymorphism (SNP) density requirements for breeding applications. A two-phase core collection strategy was then implemented to balance genetic diversity, representativeness, and experimental feasibility. In the first phase, 250 accessions were selected to enable large-scale studies such as genome-wide association studies (GWAS). This set was further refined in the second phase to 50 accessions, retaining 89.4% of allele coverage. Preliminary application of the core collection to PMMoV resistance screening identified three candidate accessions- Pcc2, Pcc55, and Pcc167- with potential resistance. Overall, this work establishes a genetic structure-informed core collection framework that enhances germplasm utilization, accelerates early-stage resistance evaluation, and provides a foundation for future genomic and breeding research in pepper.

Key words: Pepper, Genetic diversity, Core collection, Pepper mild mottle virus (PMMoV).

INTRODUCTION

Pepper (*Capsicum* spp.) is one of the most important vegetable and spice crops worldwide, valued for both its economic significance and for its nutritional compounds such as capsaicinoids and carotenoids. Breeding has targeted diverse traits- including pungency, fruit morphology,

Received: October 31, 2025; Accepted: January 28, 2026.
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stress tolerance, and disease resistance- that originate from broad germplasm resources (Nakayama *et al*. 2019; Karim *et al*. 2021). For example, anthracnose resistance in *C. annuum* has been introgressed from *C. baccatum* and *C. chinense*, illustrating the critical role of genetic resources in cultivar development (Barchenger *et al*. 2019; Srivastava & Mangal 2019). Accordingly, national and international genebanks have been established to conserve and provide access to this essential diversity (Pereira-Dias *et al*. 2019; Solomon *et al*. 2019; Tripodi *et al*. 2021).

Although genetic resources provide essential variation for crop improvement, their effective utilization remains constrained by two major challenges: the lack of genetic characterization for many accessions and the difficulty of maintaining large collections. To address these issues, Lee *et al*. (2016) analyzed 3,821 accessions representing 11 species using (SNP) markers and identified 10 genetic clusters and five phylogenetic groups. Similarly, Pereira-Dias *et al*. (2019) evaluated 190 accessions and landraces and classified them into seven clusters. Together, these studies underscore the importance of population structure analysis for the strategic use of germplasm in breeding.

To address the difficulty of maintaining and utilizing excessively large germplasm collections, Brown (1989) proposed the concept of a core collection- a strategically selected subset that captures most of the genetic diversity of the entire collection while minimizing redundancy. By condensing essential variation into a manageable sample size, core collections greatly improve the efficiency of germplasm conservation and utilization. They also provide a practical foundation for genotype-phenotype association studies and trait screening, thereby accelerating breeding progress (Balfourier *et al*. 2007; Richards *et al*. 2009; Mahmoodi *et al*. 2019).

Several core collections of pepper have been established to capture genetic diversity across species and geographic origins. Lee *et al*. (2016) selected 240 accessions from 3,821 using transcriptome-based SNPs, offering a resource for downstream association studies and gene discovery. Similarly, Nicolaï *et al*. (2013) genotyped 1,352 accessions using 28 simple sequence repeat (SSR) markers and constructed a core set of 332 entries that retained 97% of the genetic and phenotypic diversity of *C. annuum*. They also proposed nested subsets of varying sizes (8, 16, 32, 64, and 128 accessions), providing flexibility for studies with different resource constraints. This example illustrates that core collections of varying sizes can support experiments at different scales.

Pepper mild mottle virus (PMMoV) is a persistent tobamovirus that poses a serious threat to global pepper production, having been reported in more than 20 pepper-growing countries (Kumari *et al*. 2023; Ochar *et al*. 2023) and causing mottling, leaf deformation, stunting, and yield loss (Elbeshehy *et al*. 2023; Ochar *et al*. 2023). Although resistance is conferred by the *L* gene, the rapid evolution of the virus undermines the durability of these sources, creating an urgent need to identify new resistance (Mongkolporn & Taylor 2011; Barchenger *et al*. 2019; Hur *et al*. 2022). To demonstrate the practical utility of our framework, we analyzed the population structure and linkage disequilibrium of 1,011 pepper accessions, constructed a nested core collection comprising subsets of different sizes, and used PMMoV resistance screening as a case study to evaluate its application. This approach highlights how core collections of varying scales can be applied flexibly to different breeding objectives, while also identifying candidate accessions with potential resistance to PMMoV.

MATERIALS AND METHODS

Plant material and deoxyribonucleic acid (DNA) extraction

A total of 1,054 pepper accessions were used in this study, including 688 *C. annuum*, 231 *C. baccatum*, 15 *C. chinense*, 33 *C. frutescens*, 11 *C. pubescens*, 21 *C. chacoense*, 2 *C. eximium*, and one accession each of *C. galapagoense*,

C. praetermissum, and *C. tovarii*, along with 50 unclassified accessions. Three plants per accession were grown under standard greenhouse conditions, and young leaves were collected 3 wk after transplanting, flash-frozen in liquid nitrogen, and stored at -80℃. Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), quantified with the Qubit™ dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), and checked for integrity by 1% agarose gel electrophoresis.

Library preparation and sequencing

Genomic libraries were prepared using double-digest restriction site-associated DNA sequencing (ddRADseq) following Peterson *et al*. (2012) with modifications based on Kao (2016). Genomic DNA was digested with PstI-HF and MspI, ligated to barcoded adapters, PCR-amplified, and size-selected for 250–600 bp fragments using the BluePippin system (Sage Science, Beverly, MA, USA). Library quality was assessed by spectrophotometry, and equal volumes of indexed products (384 accessions per library) were pooled. Paired-end sequencing (150 bp) was performed on an Illumina NovaSeq 6000 platform (S4 flow cell).

Sequence analysis and SNP discovery

Raw sequencing data (.fastq.gz) were processed using "process_radtags" in Stacks v2.61 to demultiplex reads, remove those with incorrect enzyme sites or low quality (Phred < 20), and trim index/adaptor sequences. Clean reads were aligned to the *C. annuum* 'CM334' v1.60 reference genome (Kim *et al*. 2014) using BWA-MEM v0.7.17 (Li 2013). BAM file processing was conducted using SAMtools v1.7 (Li & Durbin 2009), and SNPs were called using the "HaplotypeCaller" module of GATK v4.2.0.0 (McKenna *et al*. 2010). SNP filtering was conducted using VCFtools v0.1.15 with the following criteria: --minDP 3, --minGQ 10, --max-missing 0.5, and --maf 0.05. Samples with an average sequencing depth < 3 (via --depth) were removed, and the remaining 1,011 accessions were used for all subsequent population structure and core collection analyses.

Population structure and genetic diversity analysis

The final SNP dataset (variant call format; VCF) was converted to genomic data structure (GDS) format using the "SNPRelate" package in R. A phylogenetic tree was generated based on the identity-by-state (IBS) matrix using hierarchical clustering and visualized with "ggtree". For population structure analysis, SNP data were converted to STRUCTURE format using PGDSpider v2.1.1.5, and STRUCTURE was run with 10,000 burn-in steps and 100,000 Markov chain Monte Carlo (MCMC) iterations for $K = 1$–9. The optimal K was determined using the Delta K method with Structure Harvester (Earl & vonHoldt 2012) and visualized in R.

Linkage disequilibrium (LD) decay was analyzed with TASSEL v5 (Bradbury *et al*. 2007), and decay curves were plotted in R. Genotypic principal component analysis (PCA) was performed in "SNPRelate", and the first two principal components were visualized with 90% confidence ellipses. Phenotypic PCA was conducted for 15 standardized traits measured in 250 core accessions; missing data were imputed with "missMDA", and PCA results were visualized in "ggplot2" with trait vectors displayed as biplot arrows.

Core collection construction

Two strategies were used to evaluate core set performance: random sampling and a hybrid A-NE/E-NE optimization using the "corehunter" package in R (De Beukelaer *et al*. 2018). Allele coverage was calculated across various sample sizes (n = 950 to 5) and visualized using LOWESS-smoothing in "ggplot2". Core collection construction was performed in two stages. In Stage 1, 250 accessions were selected from 1,011 using the A-NE/E-NE (0.5/0.5) strategy. Simulations of 1,000 subsets were run to identify accessions with the highest selection frequency. In Stage 2, stepwise nesting was

performed to create smaller core subsets (200, 150, 100, and 50 accessions), all nested within the 250-set, using the same strategy.

PMMoV resistance screening

Two inbred lines, H84 (resistant) and H15 (susceptible), were included as controls. Twenty accessions were randomly selected from the 50-accession core subset, and eight plants per accession were used, with four plants mechanically inoculated and four left uninoculated as healthy controls. At the 4–6 true-leaf stage, plants were mechanically inoculated with PMMoV by gently rubbing carborundum-dusted leaves with sap prepared from infected tissue. Disease severity was assessed 14 d post-inoculation using a four-point disease index (DI): 0 = no symptoms; 1 = mild chlorosis/mottling on new leaves; 2 = chlorosis on new and mature leaves; 3 = severe symptoms on mature leaves. PMMoV infection was confirmed by indirect enzyme-linked immunosorbent assay (ELISA) following Clark & Adams (1977) with modifications (Chen *et al.* 2022), and samples with absorbance values greater than twice those of the healthy controls were considered positive.

RESULTS

Sequencing data analysis and SNP discovery

A total of 1,011 pepper accessions were sequenced using ddRADseq, generating an average of 16.4 million reads per sample with a GC content of 41.2%. After quality filtering, 89.1% of the reads were uniquely mapped to the genome, averaging 13.5 million reads per sample. These reads covered approximately 0.5% of the genome (13 Mb). SNP discovery yielded 235,478 loci, of which 6,437 high-quality SNPs were retained after stringent filtering, corresponding to an average density of one SNP per 543,732 bp. These SNPs spanned all 12 chromosomes of *Capsicum*, providing genome-wide coverage with an average of 536 SNPs per chromosome (Fig. 1).

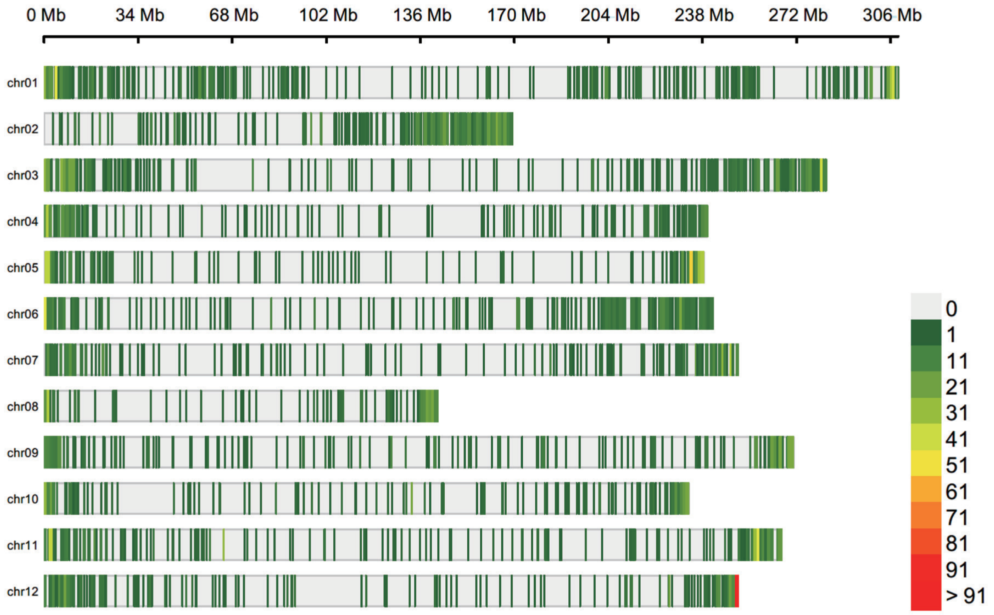

Fig. 1. Genome-wide distribution of single-nucleotide polymorphisms (SNPs) in 1,011 pepper accessions. SNP density across the 12 chromosomes is shown in 1-Mb windows. The color scale represents the number of SNPs per window, with darker green indicating lower density and red indicating higher density.

Population structure analysis

Clustering and phylogenetic analyses revealed clear population structure among the 1,011 accessions. Accessions clustered largely by species, with *C. annuum* forming the largest group, followed by *C. baccatum*, *C. frutescens*, *C. chinense*, and *C. chacoense*. Structure analysis identified four major genetic clusters (ΔK = 4; Fig. 2). Clusters 1 and 3 were composed primarily of *C. annuum*, suggesting the effects of selective breeding, while Cluster 2 included *C. baccatum*, *C. chacoense*, and some *C. pubescens*. Cluster 4 contained mostly *C. frutescens*, *C. chinense*, and a subset of *C. baccatum*. PCA based on genotypes also separated major species and clusters, with *C. annuum* and *C. baccatum* well distinguished, although *C. frutescens* and *C. chinense* showed partial overlap (Fig. 3). These

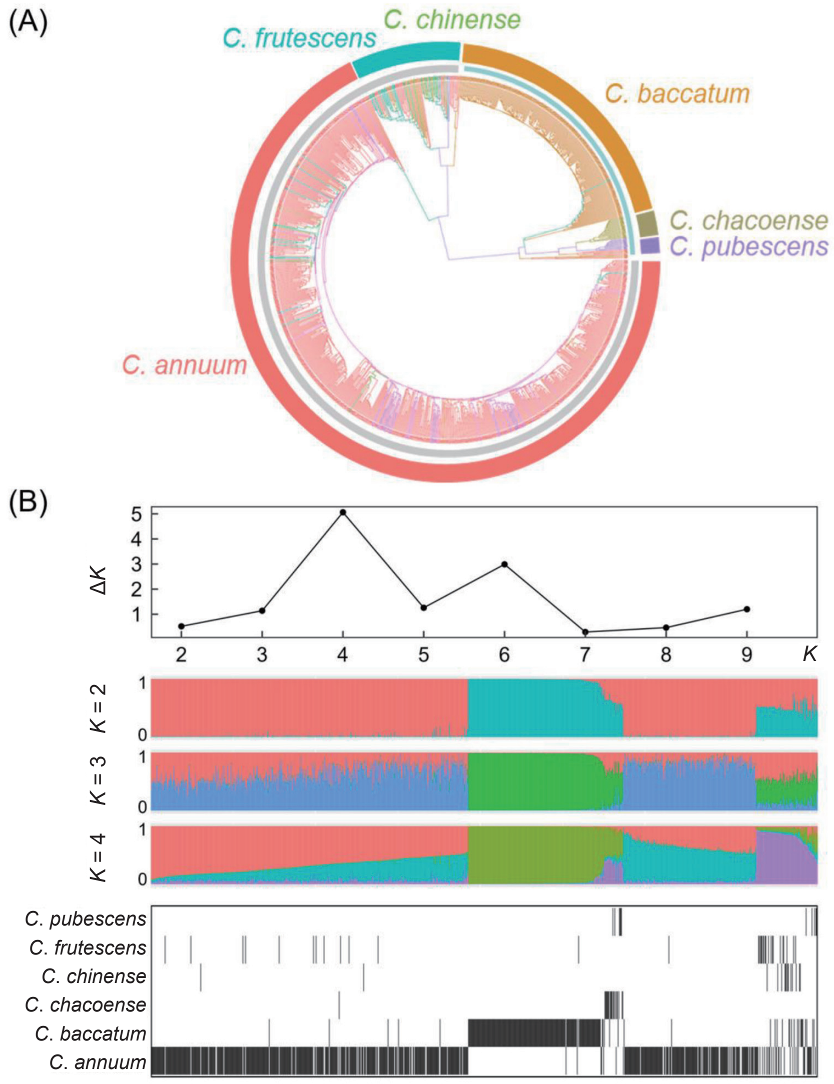

Fig. 2. Population structure of 1,011 pepper accessions. (A) Phylogenetic tree based on the identity-by-state (IBS) matrix, with major *Capsicum* species indicated by colored outer rings. (B) Population structure analysis of K = 2–4 using STRUCTURE. The Delta K plot identifies K = 4 as the optimal number of clusters, and the corresponding bar plots show admixture proportions for each accession.

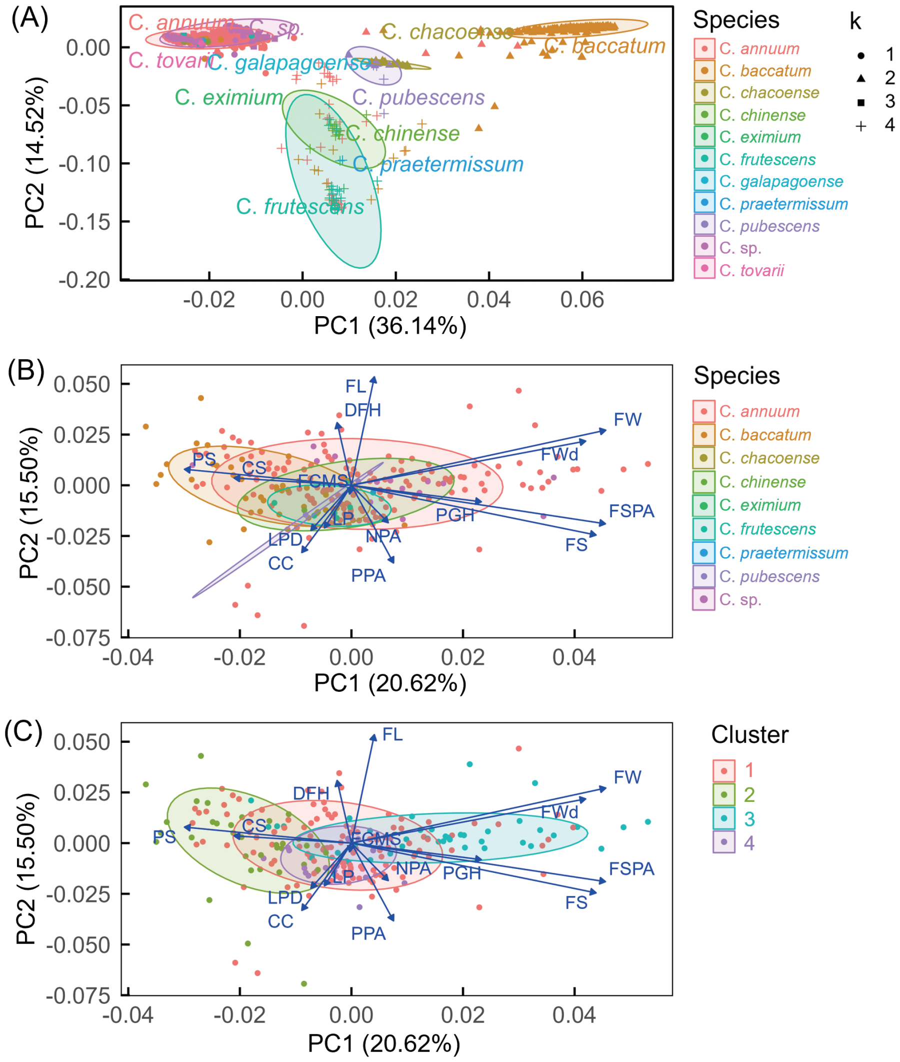

Fig. 3. Principal component analysis (PCA) of the pepper germplasm. (A) Genotypic PCA of 1,011 accessions showing separation of major *Capsicum* species with 90% confidence ellipses. (B) Phenotypic PCA of the 250-accession core set grouped by species. (C) Phenotypic PCA of the 250-accession core set grouped by genetic clusters. Trait loadings are shown as vector arrows, where arrow direction indicates correlation with the principal components and arrow length reflects contribution magnitude. The 15 traits and their abbreviations are: plant growth habit (PGH), plant stature (PS), leaf pubescence density (LPD), leaf pigmentation (LP), pedicel position at anthesis (PPA), number of pedicels per axil (NPA), corolla color (CC), corolla spot (CS), fruit color at mature stage (FCMS), fruit shape (FS), fruit shape at peduncle attachment (FSPA), fruit weight (FW), fruit length (FL), fruit width (FWd), and days from flowering to harvest (DFH).

results are consistent with previous reports (Kim *et al*. 2014).

Linkage disequilibrium decay and population analysis

To evaluate marker density requirements for genome-wide association studies (GWAS), linkage disequilibrium (LD) decay was estimated for the core collection. Across all accessions, LD decayed to an r^2 of 0.2 at 16.71 Mb, indicating that approximately 209 SNPs would be needed to cover the 3.5 Gb pepper genome (Fig. 4).

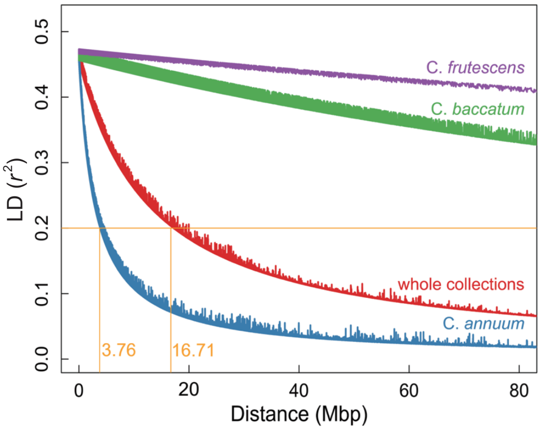

Fig. 4. Linkage disequilibrium (LD) decay in 1,011 pepper accessions. LD decay (r^2) is plotted against physical distance (Mbp) for the full set of accessions and for the three largest species (*Capsicum annuum*, *C. baccatum*, and *C. frutescens*). The horizontal line indicates the r^2 threshold of 0.2, and its intersections with the curves represent the estimated LD decay distances.

Species-specific analyses revealed notable variation: *C. annuum* showed a much shorter LD decay distance of 3.76 Mb, requiring at least 931 SNPs for genome-wide coverage, whereas LD decay thresholds for *C. baccatum* and *C. frutescens* did not reach within the tested range, likely reflecting smaller population sizes and limited recombination sampling. These patterns suggest faster LD decay in *C. annuum*, possibly due to greater recombination activity or stronger historical selection, while the slower decay in *C. baccatum* and *C. frutescens* may result from narrower genetic variation or sampling effects. Overall, the medium-density SNP dataset generated here is sufficient to support GWAS in *Capsicum*, particularly in *C. annuum*.

Construction of core collections with different sample sizes

Building on these population-level insights, we constructed nested core collections of different sizes to address diverse research needs while accommodating experimental limitations such as space and labor. Simulations showed that a 250-accession set captured 92.3% of allelic diversity, while even the smallest 50-accession set retained 89.4% coverage (Fig. 5). Based on these results, a two-phase strategy was implemented: first reducing the full set of 1,011 accessions to 250 (approximately 25%), and then generating nested subsets of 200, 150, 100, and 50 (approximately 5%), with smaller sets nested within larger ones (Table 1). Both phases used a hybrid selection strategy balancing A-NE and E-NE criteria to maximize allelic diversity while minimizing redundancy. Observed genotype coverage closely matched simulation predictions- 92.3% at 250 accessions and 89.4% at 50 accessions- and PCA biplots confirmed that the genetic structure of the subsets mirrored that of the full population (Fig. 6). These results demonstrate that the nested core collections are both representative and scalable, thereby enabling efficient resource allocation for preliminary screening and downstream applications.

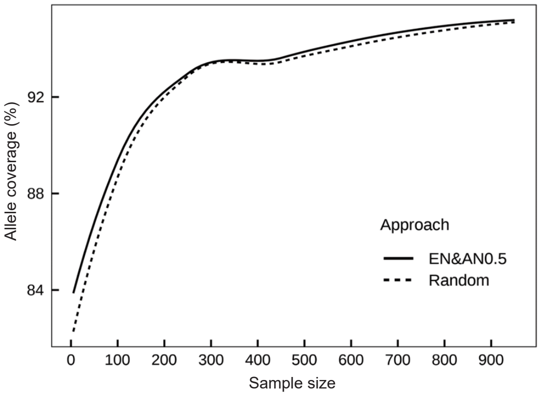

Fig. 5. Allele coverage across different core collection sample sizes. The relationship between sample size and allele coverage (%) is shown for two approaches: CoreHunter3 (solid line) and random sampling (dashed line).

Table 1. Allele coverage of pepper core collections with varying sample sizes.

No. of accessions included in the core collection	Allele coverage
250	92.3%
200	91.8%
150	91.3%
100	90.8%
50	89.4%

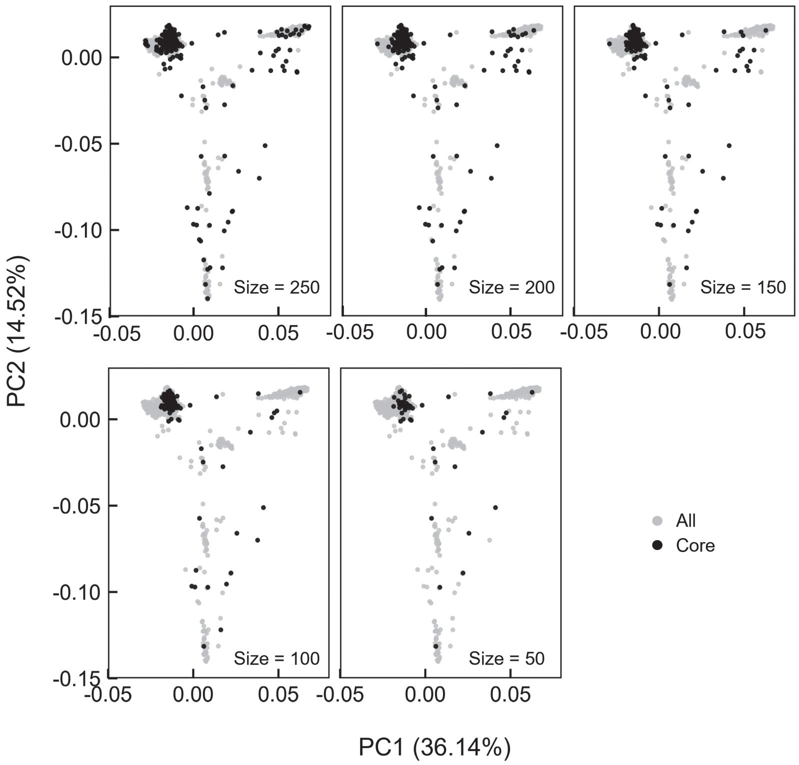

Fig. 6. Genotypic principal component analysis (PCA) of core collections with different sample sizes. PCA biplots of pepper core collections with sample sizes of 250, 200, 150, 100, and 50. Black dots indicate accessions included in each core collection, while gray dots represent the full set of 1,011 accessions.

Phenotypic trait analysis of the pepper core collection

To assess whether the core collection preserved representative phenotypic diversity, we analyzed 15 morphological and agronomic traits in the 250-accession set. In the PCA based on species (Fig. 3B), *C. annuum* displayed the broadest phenotypic variation, reflecting its large population size and domestication history. The *C. baccatum* group was distinguished by strong loadings on plant stature (PS) and corolla spot (CS), while other traits such as fruit weight (FW), fruit width (FWd), fruit shape (FS), and fruit shape at peduncle attachment (FSPA) also contributed to species-level differentiation, although partial overlap remained.

By contrast, PCA based on genetic clusters (Fig. 3C) revealed clearer phenotypic separation. Clusters 1 and 3, both largely composed of *C. annuum*, diverged along FW and FWd, consistent with distinctions between sweet and hot pepper types. Cluster 2, predominantly *C. baccatum*, separated mainly along PS and CS, in agreement with species-level classifications. These results confirm that the core collection captures key phenotypic variation across species and clusters, ensuring the retention of traits relevant to breeding objectives.

Screening of PMMoV-resistant pepper accessions

To demonstrate the practical utility of the constructed core collection in disease resistance screening, PMMoV evaluation was conducted as a case study. Due to the limited capacity of the inoculation platform, which restricted the number of accessions that could be handled simultaneously, 20 *C. annuum*

accessions were randomly selected from the 50-accession core set for evaluation.

The resistant control H84 exhibited low disease index (DI = 0.75 ± 0.96) and ELISA absorbance (0.90 ± 1.06) at 14 d post-inoculation, whereas the susceptible control H15 consistently showed high DI and ELISA values (2.31 ± 0.81 and 2.50 ± 0.58, respectively), confirming the reliability of the assay (Table 2, Fig. 7). Among the tested accessions, Pcc2 (GeneBank ID: 2023A01067) displayed the lowest DI (0.25 ± 0.50) and ELISA value (0.06 ± 0.09), indicating potential resistance to PMMoV. Two additional accessions, Pcc55 (DI = 0.50 ± 0.58; ELISA = 0.70 ± 0.78) and Pcc167 (DI = 1.00 ± 0.82; ELISA = 0.24 ± 0.46), also showed reduced symptom severity and viral accumulation, suggesting their potential as candidate sources of resistance.

Table 2. Resistance analysis of pepper core collection against pepper mild mottle virus (PMMoV).

Name	GeneBank ID	ELISA[z]	DI[y]
H84 (RC[x])	-	0.90 ± 1.06	0.75 ± 0.96
H15 (SC[w])	-	2.31 ± 0.81	2.50 ± 0.58
Pcc2	2023A01067	0.06 ± 0.09	0.25 ± 0.50
Pcc5	2023A01069	0.87 ± 0.70	1.75 ± 1.26
Pcc16	2024A00467	1.29 ± 1.24	1.75 ± 0.96
Pcc53	2023A01139	1.65 ± 1.41	2.50 ± 0.58
Pcc55	2023A01141	0.70 ± 0.78	0.50 ± 0.58
Pcc60	2023A01144	0.25 ± 0.16	1.75 ± 0.96
Pcc63	2023A01147	0.44 ± 0.73	2.00 ± 1.41
Pcc65	2023A01149	0.45 ± 0.73	1.50 ± 1.29
Pcc69	2023A01153	0.77 ± 0.80	1.50 ± 1.29
Pcc72	2023A01156	1.47 ± 1.67	0.75 ± 0.96
Pcc84	2023A01164	1.13 ± 1.36	2.00 ± 0.82
Pcc89	2023A01168	1.74 ± 1.09	1.00 ± 0.82
Pcc91	2023A01169	0.44 ± 0.32	1.50 ± 0.58
Pcc99	2024A00401	1.26 ± 1.30	2.75 ± 0.50
Pcc105	2023A01180	0.88 ± 0.52	2.00 ± 0.82
Pcc146	2023A01213	0.24 ± 0.30	1.50 ± 0.58
Pcc159	2023A01120	1.50 ± 1.01	1.50 ± 1.29
Pcc167	2023A01128	0.24 ± 0.46	1.00 ± 0.82
Pcc168	2023A01129	0.34 ± 0.57	1.25 ± 0.96
Pcc197	2023A01117	1.65 ± 1.25	1.75 ± 0.96

[z] ELISA: enzyme-linked immunosorbent assay.
[y] Disease index (DI) expressed as mean ± standard deviation, measured 14 d post-inoculation.
[x] RC: resistant control.
[w] SC: susceptible control.

DISCUSSION

This study combined genome-wide SNP discovery with population structure and phenotypic analyses to develop nested core collections of pepper. By integrating genomic and phenotypic diversity, the collections provide both a strategic framework for germplasm conservation and practical resources for trait-based breeding.

In this study, a total of 1,011 primary pepper core accessions were genotyped using the ddRADseq, yielding 6,437 high-quality SNP markers distributed across all 12 chromosomes. These markers serve as foundational genomic tools for *Capsicum* research, enabling refined analyses of genetic diversity, species classification, and gene discovery. Population structure analysis revealed clear population differentiation, particularly within *C. annuum*, where gradual transitions between clusters likely reflect historical selection for sweet and chili types. Integration of clustering results with phenotypic data further supported this distinction, highlighting the utility of genetic structure information in guiding parent selection for trait-oriented breeding.

In contrast, species such as *C. baccatum* and *C. frutescens* exhibited more homogeneous genetic profiles, suggesting reproductive isolation or less intensive selection. Nevertheless, several admixed accessions were identified, representing promising materials for interspecific introgression, especially for the transfer of disease resistance traits.

LD decay patterns also have important implications for breeding strategies. The rapid decay observed in *C. annuum* indicates its suitability for high-resolution GWAS, but emphasizes the need for dense marker coverage and precise phenotyping. By contrast, the slower decay in *C. baccatum* and *C. frutescens* suggests that lower marker densities may suffice for trait mapping in these species, which

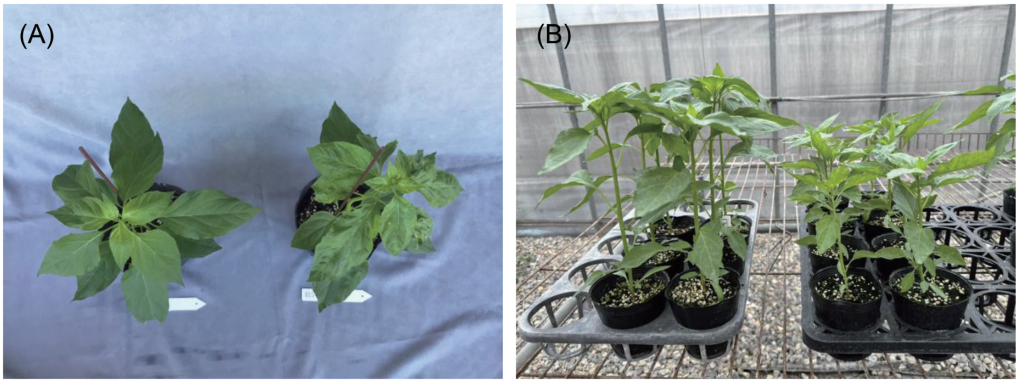

Fig. 7. Symptoms caused by pepper mild mottle virus (PMMoV) on the susceptible control H15 at 14 d post-inoculation. (A) Mosaic symptoms observed in inoculated plants (right) compared with non-inoculated controls (left). (B) Stunting was observed in inoculated plants (right) compared with non-inoculated controls (left).

could reduce genotyping costs. Together, these insights help optimize marker-assisted strategies across different pepper gene pools, particularly when introgressing traits from wild or minor species.

To address practical limitations in germplasm evaluation, we developed a nested core collection consisting of subsets of 250, 200, 150, 100, and 50 accessions. This stepwise design balances allele diversity, representativeness, and scalability, ensuring that researchers can select a core size appropriate to their experimental capacity. Even the smallest subsets preserved most of the genetic and phenotypic variation, demonstrating that compact cores can still serve as effective proxies for the full population. Importantly, phenotypic analyses confirmed that key agronomic distinctions, such as those between hot and sweet *C. annuum*, were retained, underscoring the value of the cores for both germplasm conservation and trait-based selection.

To illustrate the utility of the nested core collection under practical breeding constraints, the PMMoV assay in this study was intentionally designed as an early-stage, exploratory screening rather than as a definitive resistance validation. Accordingly, result interpretation focused on overall trends and relative performance among accessions, rather than on strict hypothesis testing.

Although differences between the resistant (H84) and susceptible (H15) controls did not reach statistical significance, the observed mean differences in both DI, reflecting external symptom expression, and ELISA-based measurements of viral accumulation were directionally consistent with their established resistance phenotypes. The lack of statistical significance is most plausibly attributable to limited replication (four inoculated plants per line), together with variability inherent to mechanical inoculation and the distinct measurement characteristics of DI and ELISA.

DI and ELISA capture complementary but non-equivalent aspects of host-virus interactions. DI represents symptom severity on an ordinal scale with constrained resolution and potential ceiling effects, whereas ELISA quantifies viral accumulation at the tissue level and is inherently more sensitive to quantitative variation. Consequently, greater variability in ELISA relative to DI is expected in early-stage screening assays, particularly for accessions exhibiting partial resistance or tolerance, where symptom expression does not necessarily scale linearly with viral load.

Within this framework, accessions were compared with the resistant and susceptible controls, focusing on those with relatively low DI scores and reduced viral accumulation.

Under these conditions, Pcc2, Pcc55, and Pcc167 were among the accessions exhibiting this response pattern. However, these results should be interpreted with caution, and further evaluation with increased replication and repeated inoculation trials is required before any conclusion can be made regarding their resistance.

Overall, this study presents a strategic framework for constructing and applying nested pepper core collections based on genetic structure and phenotypic diversity. The established collections enhance the efficiency of trait screening and breeding while contributing to the long-term conservation and utilization of genetic resources. The developed pepper core collection can be applied to support marker-assisted selection, GWAS, and the development of elite cultivars under practical breeding constraints.

CONCLUSIONS

This study developed a nested core collection of pepper based on genome-wide SNPs and population structure, providing a scalable resource that balances genetic diversity, representativeness, and experimental feasibility. Validation with phenotypic traits and a case study on PMMoV resistance confirmed its potential for efficient trait screening. The developed core sets offer a practical tool for conserving genetic diversity and can be readily applied to support breeding programs, marker-assisted selection, and genome-wide studies under practical constraints.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Agriculture for funding support through projects 110AS-4.6.1-CI-C9 and 111AS-4.6.1-CI-C7. The pepper germplasm used in this study was kindly provided by the National Plant Genetic Resources Center of the Taiwan Agricultural Research Institute and the Genebank of World Vegetable Center. We appreciate their generous contributions, which were essential for the success of this research.

REFERENCES

Balfourier, F., V. Roussel, P. Strelchenko, F. Exbrayat-Vinson, P. Sourdille, G. Boutet, ... G. Charmet. 2007. A worldwide bread wheat core collection arrayed in a 384-well plate. Theor. Appl. Genet. 114:1265–1275. doi:10.1007/s00122-007-0517-1

Barchenger, D. W., P. Naresh, and S. Kumar. 2019. Genetic resources of *Capsicum*. p.9–23. *in*: The *Capsicum* Genome. (Ramchiary, N. and C. Kole, eds.) Springer. Cham, Switzerland. 232 pp. doi:10.1007/978-3-319-97217-6_2

Bradbury, P. J., Z. Zhang, D. E. Kroon, T. M. Casstevens, Y. Ramdoss, and E. S. Buckler. 2007. TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635. doi:10.1093/bioinformatics/btm308

Brown, A. H. D. 1989. Core collections: A practical approach to genetic resources management. Genome 31:818–824. doi:10.1139/g89-144

Chen, C. C., F. L. Chiang, Y. H. Cheng, and J. Y. Liao. 2022. Improvement of wide-spectrum detection of different potyviruses infecting passion fruit by the mixture of two kinds of polyclonal antibodies against east Asian passiflora virus. J. Taiwan Agric. Res. 71:101–121. (in Chinese with English abstract) doi:10.6156/JTAR.202206_71(2).0002

Clark, M. F. and A. N. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475–483. doi:10.1099/0022-1317-34-3-475

De Beukelaer, H., G. F. Davenport, and V. Fack. 2018. Core Hunter 3: Flexible core subset selection. BMC Bioinformatics 19:203. doi:10.1186/s12859-018-2209-z

Earl, D. A. and B. M. vonHoldt. 2012. Structure Harvester: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4:359–361. doi:10.1007/s12686-011-9548-7

Elbeshehy, E. K. F., W. M. Hassan, and A. A. Baeshen. 2023. Controlling pepper mild mottle virus (PMMoV) infection in pepper seedlings by use of chemically synthetic silver nanoparticles. Molecules 28:139. doi:10.3390/molecules28010139

Hur, O. S., H. R. Kwak, N. Y. Ro, Y. Choi, S. Lee, A. Hwang, ... B. S. Hahn. 2022. Resistance screening of *Capsicum* germplasm to pepper mild mottle virus (PMMoV) pathotypes $P_{1,2}$ and $P_{1,2,3}$. Korean J. Breed. Sci. 54:1–7. (in Korean with English abstract) doi:10.9787/KJBS.2022.54.1.1

Kao, C. 2016. Construction of a high density linkage map

with double digest RADseq and mapping of restorer-of-fertility gene in pepper (*Capsicum annuum* L.). Master Thesis. Department of Agronomy, National Taiwan University. Taipei, Taiwan. 94 pp. (in Chinese with English abstract) doi:10.6342/NTU201610013

Karim, K. M. R., M. Y. Rafii, A. B. Misran, M. F. B. Ismail, A. R. Harun, M. M. H. Khan, and M. F. N. Chowdhury. 2021. Current and prospective strategies in the varietal improvement of chilli (*Capsicum annuum* L.) specially heterosis breeding. Agronomy 11:2217. doi:10.3390/agronomy11112217

Kim, S., M. Park, S. I. Yeom, Y. M. Kim, J. M. Lee, H. A. Lee, … D. Choi. 2014. Genome sequence of the hot pepper provides insights into the evolution of pungency in *Capsicum* species. Nat. Genet. 46:270–278. doi:10.1038/ng.2877

Kumari, N., V. Sharma, P. Patel, and P. N. Sharma. 2023. Pepper mild mottle virus: A formidable foe of *Capsicum* production- A review. Front. Virol. 3:1208853. doi:10.3389/fviro.2023.1208853

Lee, H. Y., N. Y. Ro, H. J. Jeong, J. K. Kwon, J. Jo, Y. Ha, … B. C. Kang. 2016. Genetic diversity and population structure analysis to construct a core collection from a large *Capsicum* germplasm. BMC Genet. 17:142. doi:10.1186/s12863-016-0452-8

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997. doi:10.48550/arXiv.1303.3997

Li, H. and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. doi:10.1093/bioinformatics/btp324

Mahmoodi, R., M. R. Dadpour, D. Hassani, M. Zeinalabedini, E. Vendramin, S. Micali, and F. Z. Nahandi. 2019. Development of a core collection in Iranian walnut (*Juglans regia* L.) germplasm using the phenotypic diversity. Sci. Hortic. 249:439–448. doi:10.1016/j.scienta.2019.02.017

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, … M. A. DePristo. 2010. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20:1297–1303. doi:10.1101/gr.107524.110

Mongkolporn, O. and P. W. J. Taylor. 2011. *Capsicum*. p.43–57. *in*: Wild Crop Relatives: Genomic and Breeding Resources. (Kole, C., ed.) Springer. Heidelberg, Germany. 282 pp. doi:10.1007/978-3-642-20450-0_4

Nakayama, T., S. Takahashi, and T. Waki. 2019. Formation of flavonoid metabolons: Functional significance of protein-protein interactions and impact on flavonoid chemodiversity. Front. Plant Sci. 10:821. doi:10.3389/fpls.2019.00821

Nicolaï, M., M. Cantet, V. Lefebvre, A. M. Sage-Palloix, and A. Palloix. 2013. Genotyping a large collection of pepper (*Capsicum* spp.) with SSR loci brings new evidence for the wild origin of cultivated *C. annuum* and the structuring of genetic diversity by human selection of cultivar types. Genet. Resour. Crop Evol. 60:2375–2390. doi:10.1007/s10722-013-0006-0

Ochar, K., H. C. Ko, H. J. Woo, B. S. Hahn, and O. Hur. 2023. Pepper mild mottle virus: An infectious pathogen in pepper production and a potential indicator of domestic water quality. Viruses 15:282. doi:10.3390/v15020282

Pereira-Dias, L., S. Vilanova, A. Fita, J. Prohens, and A. Rodríguez-Burruezo. 2019. Genetic diversity, population structure, and relationships in a collection of pepper (*Capsicum* spp.) landraces from the Spanish centre of diversity revealed by genotyping-by-sequencing (GBS). Hortic. Res. 6:54. doi:10.1038/s41438-019-0132-8

Peterson, B. K., J. N. Weber, E. H. Kay, H. S. Fisher, and H. E. Hoekstra. 2012. Double digest RADseq: An inexpensive method for *de novo* SNP discovery and genotyping in model and non-model species. PLoS One 7:e37135. doi:10.1371/journal.pone.0037135

Richards, C. M., G. M. Volk, P. A. Reeves, A. A. Reilley, A. D. Henk, P. L. Forsline, and H. S. Aldwinckle. 2009. Selection of stratified core sets representing wild apple (*Malus sieversii*). J. Amer. Soc. Hortic. Sci. 134:228–235. doi:10.21273/JASHS.134.2.228

Solomon, A. M., K. Han, J. H. Lee, H. Y. Lee, S. Jang, and B. C. Kang. 2019. Genetic diversity and population structure of Ethiopian *Capsicum* germplasms. PLoS One 14:e0216886. doi:10.1371/journal.pone.0216886

Srivastava, A. and M. Mangal. 2019. Capsicum breeding: History and development. p.25–55. *in*: The Capsicum Genome. (Ramchiary, N. and C. Kole, eds.) Springer. Cham, Switzerland. 232 pp. doi:10.1007/978-3-319-97217-6_3

Tripodi, P., M. T. Rabanus-Wallace, L. Barchi, S. Kale, S. Esposito, A. Acquadro, … N. Stein. 2021. Global range expansion history of pepper (*Capsicum* spp.) revealed by over 10,000 genebank accessions. Proc. Natl. Acad. Sci. U.S.A. 118:e2104315118. doi:10.1073/pnas.2104315118

基於遺傳結構之番椒核心種原建立與應用

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摘要

李承彬、王昭月、林大鈞、陳金枝、林靜宜、杜元凱、關政平、林思妤。2026。基於遺傳結構之番椒核心種原建立與應用。台灣農業研究 75(2):351–363。

本研究旨在建立以遺傳結構為基礎之番椒 (*Capsicum* spp.) 核心種原，以促進遺傳資源之保存與應用。本研究自國家種原庫蒐集 1,011 份番椒材料，採用雙重酶切 DNA 定序技術進行基因體分析，共獲得 6,437 個高品質單核苷酸多型性位點。族群結構分析顯示番椒種原可分為四大遺傳族群，其基因型與外表型變異具高度對應關係，反映族群遺傳結構與性狀表現之關聯性。進一步之連鎖不平衡分析顯示番椒種間之基因體分化特徵，可作為後續全基因體關聯性研究之分子標誌密度參考。基於此些遺傳背景資訊，本研究採雙階段策略建立番椒核心種原，首先建立涵蓋 250 個種原之番椒核心種原，再逐步縮減為涵蓋 200、150、100 及 50 個種原之番椒核心種原，此些核心種原保留 89.4% 以上之等位基因多樣性。為驗證核心種原之實用性，本研究將番椒核心種原應用於番椒微斑病毒 (pepper mild mottle virus; PMMoV) 抗性評估，結果顯示 Pcc2、Pcc55 與 Pcc167 等種原具抗病潛力。本研究建立之番椒核心種原為番椒遺傳資源之保存與應用奠定新的基礎，亦可作為後續抗逆境篩選與全基因體關聯分析等研究之重要材料。

關鍵詞：番椒、基因多樣性、核心種原、番椒微斑病毒。

投稿日期：2025 年 10 月 31 日；接受日期：2026 年 1 月 28 日。

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台灣農業研究

第七十五卷　第二期　　　　中華民國一一五年六月三十日

出版者	農業部農業試驗所
發行人	王仕賢
總編輯	石憲宗
地址	413008 臺中市霧峰區萬豐里中正路 189 號
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線上投審稿系統	https://www.ipress.tw/J0042
	台灣農業研究之稿約格式內容，詳自農業部農業試驗所首頁下列路徑下載：文宣出版 > 台灣農業研究 > 出版品稿約

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電話	(02)2926-6006
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定價	新臺幣一七五元
展售門市	1. 國家書店／ 104472 臺北市松江路 209 號 1 樓 電話 : (02)2518-0207 國家網路書店 http://www.govbooks.com.tw 2. 五南文化廣場／ 400002 臺中市中山路 6 號 電話 : (04)2226-0330

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

Vol. 75 No. 2 June 30, 2026

Published by Taiwan Agricultural Research Institute, Ministry of Agriculture

Publisher	Dennis Wang
Editor-in-Chief	Hsien-Tzung Shih
Address	No.189, Zhongzheng Rd., Wufeng Dist., Taichung City 413008, Taiwan, ROC
Website	https://www.tari.gov.tw/jtar; E-mail: jtar@tari.gov.tw; Tel: +886-4-2331-7236
Submission	https://www.ipress.tw/J0042
	Complete instructions to authors are available on the Journal of Taiwan Agricultural Research website: https://www.tari.gov.tw/jtar/
Press	Ainosco Press
Address	18F, No.80, Sec.1, Chenggong Rd., Yonghe Dist., New Taipei City 234634, Taiwan, ROC
Tel	+886-2-2926-6006
E-mail	press@airiti.com
Price	NT$ 175 (per copy)
Distributor	1. GB Books / 1F., No.209, Songjiang Rd., Taipei City 104472, Taiwan, ROC GB Website: http://www.govbooks.com.tw; Tel: +886-2-2518-0207 2. Wunan Books / No.6, Zhongshan Rd., Taichung City 400002, Taiwan, ROC; Tel: +886-4-2226-0330